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ON THE ORIGIN OF THE BROAD RAY IN QUERCUS

CONTRIBUTION FROM THE PHANEROGAMIC LABORATORY OF HARVARD UNIVERSITY NO. 17

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(WITH PLATES VIII AND IX)

As is shown in KNY'S *Botanische Wandtafeln*, nos. 75 and 76, the wood of Quercus consists of vessels, tracheids, fibers, and parenchyma. The last is disposed in two systems: the vertical, consisting of more or less scattered rows of cells, forming the xylem parenchyma; and the horizontal, made up of plates of cells extending radially, the medullary rays. The latter are of two sorts: small, numerous rays, which are thin sheets of tissue, linear, and of a single row of cells as seen in transverse and tangential sections; and occasional broad, generally fusiform masses, many cells wide, which form the most prominent feature of oak wood, its remarkable "silver grain." In our first two figures both kinds of rays are seen: *fig. 1* shows in transverse section a portion of a large ray, and of several small ones from the wood of *Quercus rubra;* and *fig. 2*, the tangential view of similar rays in the same species.

In the study of sections of a fossil oak from the gold gravels of California (Miocene), the material for which was kindly loaned the Phanerogamic Laboratories of Harvard University for investigation, an unusual ray structure was noticed. The uniseriate rays are present as in the living oaks; the large rays, however, are not homogeneous masses of parenchyma, but are represented by groups of smaller rays. *Fig. 6* shows such a group in tangential view. The smaller rays are so arranged as to form, in aggregate, the elongate fusiform mass of the normal large ray. They are separated from each other, however, by fibers, or by fibers and wood parenchyma.

A portion of the cluster more highly magnified is shown in *fig. 10*. *Fig. 5* shows the appearance of this "false" ray in transverse section.

This structure led to the suspicion that the large rays of the existent oaks might be derived from the so-called "false rays" of the lower Cupuliferae; that is, that the former have been "built up" by the aggregation and fusion of the small rays. The Miocene fossil would then represent a comparatively recent ancestral condition in ray structure. Two American species of Quercinium from Tertiary deposits in the Yellowstone National Park, *Quercinium Knowltonii* Felix[1] and *Q. lamarense* Knowlton,[2] have rays which seem, from the figures and descriptions, to be very much like those of the fossil here figured. The question then arose whether the seedlings of our living species of Quercus do not present some evidence as to the origin of the larger rays in living oaks, inasmuch as the seedling is known to be a seat of ancestral characters. The investigation of a number of North American species showed that we have in the oaks a good example of seedling recapitulation.

The oaks, from paleobotanical evidence, seem to have descended from ancestors that resembled the living chestnuts. There is much similarity in the structure of the reproductive organs, and in the type of leaf shape of the oldest oaks, and of the chestnuts. Confirmatory evidence is presented by the seedlings of some of our living oaks; especially worthy of notice is the fact that the first rays formed are all of the type which is found throughout chestnut wood.

In respect to ray structure in the seedlings, the species examined fall into two groups, corresponding with the subgenera Lepidobalanus (white oaks), and Erythrobalanus (red or black oaks).

The seedlings of the black oaks that were examined by the writer (*Q. rubra* L., *Q. velutina* Lam., *Q. coccinea* Moench., and *Q. ilicifolia* Wang.) all show a ray structure very much like that of the gold gravel oak above described. In the early annual rings, from the first or third to about the fifteenth, a progressive compounding, that is grouping and fusion, of the linear rays occurs, with the final production, after the considerable period of compounding, of a single,

[1] FELIX, J., Untersuchung über fossile Hölzer. Zeit. Deutsch. Geol. Gesells. ——:250. *pl. 6.* 1896.

[2] KNOWLTON, F. H., Geology of the Yellowstone National Park. Part II. U. S. Geol. Survey. p. 771. *pls. 118, 120, 121.* 1899.

huge, homogeneous ray. *Fig. 3* shows this process in a seedling of *Q. velutina*, the partial formation of three rays being evident. The ultimate shape and structure is brought about by the loss of the separating rows of fibers, tracheids, or wood parenchyma, or rather by the transformation of the elements included by the rays as they approach one another. This change is gradual; more and more parenchyma is formed, with corresponding decrease in fibers, until the cambial cells that lay down these radial rows cut off only ray cells. Ray formation of this sort exists in these species only as a passing phase in the seedling. In the wood of the mature plants large rays, of course, are continually formed, but their origin is nearly always abrupt. The transition from lignified elements to parenchyma occurs generally within the width of a single annual ring, or sometimes two or three. Later there is usually an increase in width which is due, however, not to the uniting of any neighboring small rays and the transformation of adjacent elements, but to simple growth in size with the increase of the stem in diameter. The seedlings of the black oaks, then, give evidence of the mode of origin of the broad rays so characteristic of the mature wood of the group. In so doing they repeat the ray structure that probably occurred throughout the wood of their rather recent ancestors.

Among the white oaks, seedlings of *Q. alba* L., *Q. bicolor* Willd., and *Q. prinoides* Willd. were examined. In all three species the wood of the stem for a distance of several inches, or even one or two feet, above the root possesses only uniseriate rays. Moreover, until the plant has attained a considerable size no large rays nor any signs of grouping of the small rays appear. Cases were seen in *Q. alba* and *Q. bicolor*, where in seedlings 15 to 20 years old only the linear rays existed. This condition would suggest that these white oaks, perhaps, are somewhat more primitive than the black oaks, for the seedlings of the former revert in structure to an ancestral type in which only uniseriate rays existed, and had not as yet begun to aggregate. *Fig. 7* shows a transverse view of this condition in *Q. alba* in about the fifteenth to twentieth annual rings. When the large rays do appear in these species, they are formed as in the black oaks, rather abruptly, the phase of compounding being confined to only one or two annual rings. Often nearly the whole ray arises at

once at the beginning of a season's growth; a continuous row of cambial cells which have hitherto laid down chiefly fibers and tracheids, beginning with the first divisions of the year, form ray cells and only such thereafter. This is also the method in the stem of the mature plant.

Among the living oaks there are exceptions, however, to the possession of rays of the type shown in *figs. 1* and *2*. *Q. virginiana* Mill. (*Q. virens* Ait.), the live oak, has large rays of a type in which the compounding has become nearly complete. This species is probably somewhat more primitive than the above-mentioned species. *Fig. 8* shows a tangential section of a ray from near the pith in a large seedling of this plant. The structure of this ray very much resembles that of the fossil oak and of the black oak seedlings. In *fig. 9* a portion of this ray is shown enlarged. The Japanese oaks have somewhat similar structure of the broad medullary rays, *Q. Mirbeckii* and *Q. Sieboldii* especially. In the shape of their leaves the oaks of Japan show other probable signs of primitiveness; the general type is similar to that of the chestnuts.

Not only does the large ray in its primitive condition occur in fossil and seedling oaks, and occasionally in mature plants of the genus, but it exists also in several genera of the lower Cupuliferae, namely in Corylus, Carpinus, Ostrya, Betula, Alnus, and perhaps others. The distribution of the large rays in the dicotyledons is at present being investigated in this laboratory by Mr. I. W. BAILEY. *Figs. 11* and *12*, transverse and tangential sections, respectively, of the wood of *Alnus incana* (L.) Moench., show in one of these genera this "false ray." In this case the linear rays have become grouped together, but very little fusion has yet taken place. It would seem then that this fusion of rays, perhaps for the acquisition of greater storage capacity, which began in some primitive cupuliferous plants, and which is still maintained at an early stage in some of the living members thereof, has reached a culmination in the broad, solidly parenchymatous mass, the large ray, of many living oaks.

That the "false ray" of Alnus and of other closely allied genera represents the primitive condition of the present large ray of Quercus seems undoubted, not only from its appearance in the lower Cupuliferae, and in fossil and seedling oaks, but also from many other points

of resemblance. A prominent feature of the broad ray of Quercus is the strong "dipping in" of the annual ring wherever these radial masses of parenchyma occur. This is well shown in *fig. 1*. In the cases among the lower Cupuliferae, and in fossil species of Quercus where rays of the compound type exist, the same feature is noticeable. In Alnus, as appears in *fig. 11*, this condition is prominent; in the fossil oak it can be seen, but only faintly, for the annual rings are not strongly marked, probably as a result of the milder climate of the Miocene. This peculiarity serves thus further to connect the two kinds of rays.

In view, then, of the apparent phylogenetic relation of the two sorts of medullary rays found in Quercus, the designation of the broad ray as *primary* and of the small one as *secondary*, a use that is to some extent prevalent, seems inadmissible. The terms would certainly be more applicable if interchanged, but the adjectives *small* and *broad* or *large*, or *small* and *compounded* may perhaps be more appropriately applied.

Summary

The medullary rays of the oak are of two sorts: small, linear sheets of parenchyma, only a single row of cells wide, as seen in transverse and tangential sections; and large, generally fusiform masses, many cells in width. The former have been to some extent called secondary rays, the latter primary rays.

Fossil evidence points to the probable derivation of the existent oaks from ancestors which possessed only the linear type of ray. The large ray has apparently originated by the aggregation and fusion of many of these small rays, through the loss of the separating elements, or the transformation of the latter into ray cells. A fossil oak from the Miocene gold gravels of California shows an intermediate condition, a stage where all the large rays consist merely of aggregations of small rays, with separating fibers. The seedlings of some, at least, of the black oaks form rays of this nature for a brief period, usually during the first ten to fifteen years. There is during this time a recapitulation of the history of the development of the ray; the small, uniseriate rays, which are at first formed, gradually approach each other in groups, and fusion slowly occurs

as growth of the stems continues, until a good-sized, homogeneous, parenchymatous ray is formed. In the wood of the mature plant the large ray is formed more abruptly, and is not so much the result of fusion of smaller rays, as of the transformation of all tissue, within a region of the size of the ray to be formed, into ray parenchyma, such change being rather abrupt and occurring largely at the beginning of a season's growth. The seedlings of the white oaks possess in their early wood only uniseriate rays, suggesting a somewhat more primitive state. A few living oaks show the transitional stage, that is, large rays composed of ununited aggregations of small rays in the wood of mature plants. The Japanese oaks especially belong in this group.

The oaks present an excellent case of recapitulation of ancestral characters by seedlings. The phylogenetic history of ray structure in the genus is well displayed, step by step, in the wood of the seedling stem during the first few years.

The work has been carried on in the Phanerogamic Laboratories of Harvard University. The writer desires to express his thanks to Dr. F. H. KNOWLTON of the United States Geological Survey for the loan of material of miocene oaks, and to Mr. I. W. BAILEY for opportunity to examine and use slides of several genera of cupuliferous woods. He is also very much indebted to Professor E. C. JEFFREY for suggestions and advice during the progress of the investigation.

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EXPLANATION OF PLATES VIII AND IX

PLATE VIII

FIG. 1.—*Quercus rubra;* transverse section of wood, showing portions of large and small medullary rays. ×40.

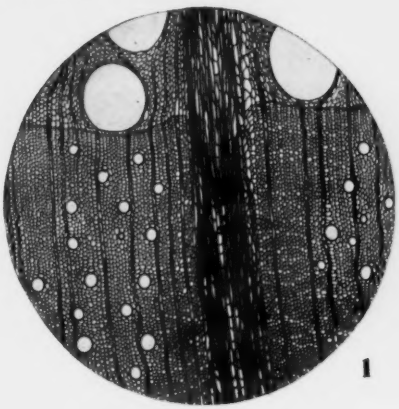
FIG. 2.—The same; tangential section of wood, showing similar rays. ×40.

FIG. 3.—*Quercus velutina*, seedling; transverse section of portion of stem showing formation of large rays. ×40.

FIG. 4.—*Quercus rubra*, seedling; tangential section of a large ray in process of formation. ×40.

FIG. 5.—Fossil oak from gold gravels of California (Miocene); transverse section of wood showing portion of the large ray. ×40.

FIG. 6.—The same; tangential view of portion of the large ray. ×40.



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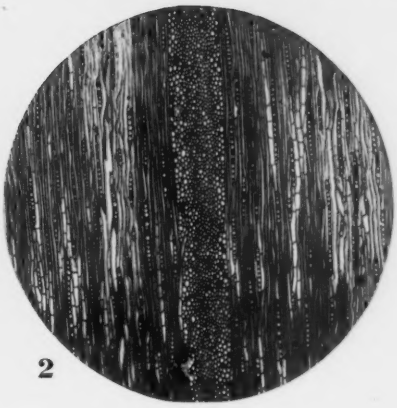

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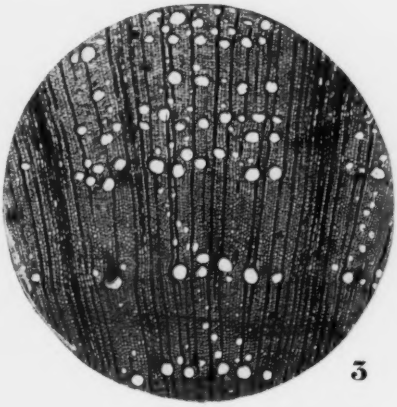

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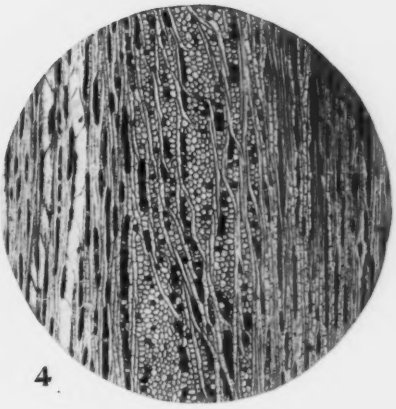

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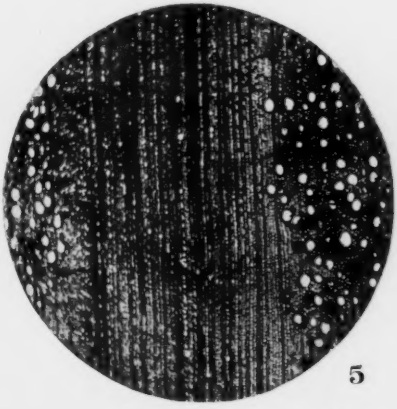

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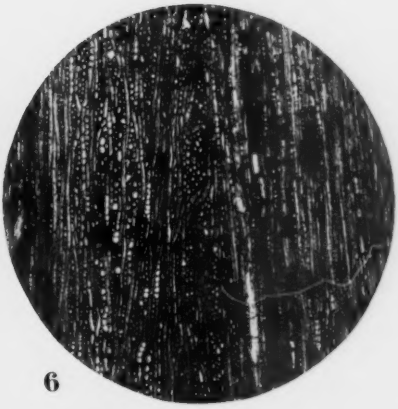

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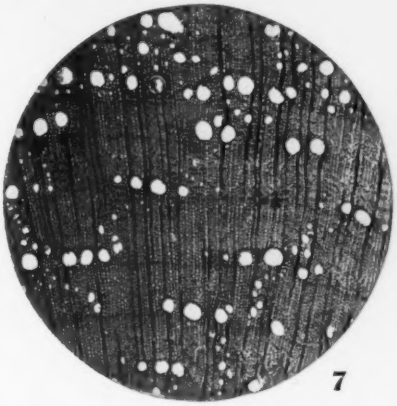
EAMES on QUERCUS



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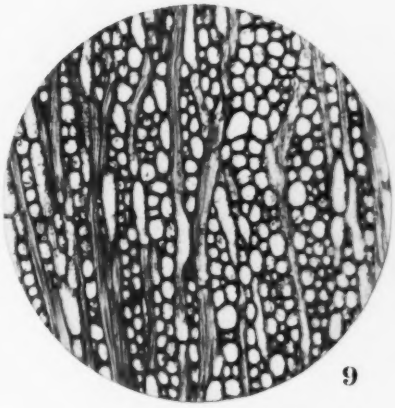

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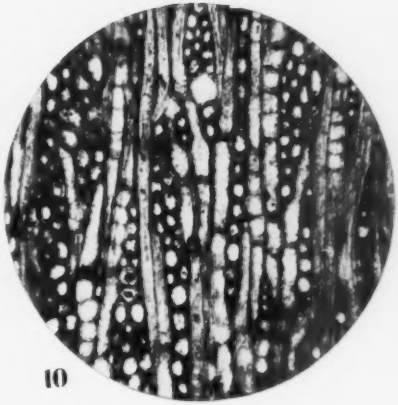

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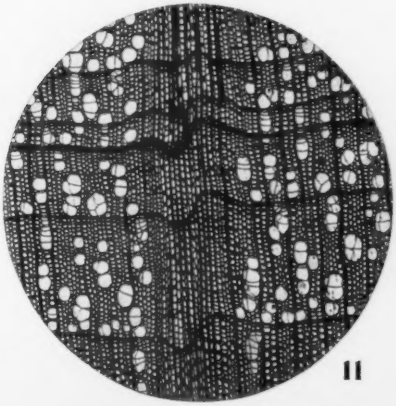

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EAMES on QUERCUS

PLATE IX

FIG. 7.—*Quercus alba*, seedling; transverse section of portion of stem, showing only rays of the linear type. ×40.

FIG. 8.—*Quercus virginiana*, seedling; tangential view of portion of large ray. ×40.

FIG. 9.—The same; portion of the large ray. ×100.

FIG. 10.—Fossil oak of the gold gravels; tangential view of portion of the large ray. ×100.

FIG. 11.—*Alnus incana;* transverse section of wood. ×40.

FIG. 12.—The same; tangential section. ×40.

THE PROTHALLIA OF ANEIMIA AND LYGODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 136

EDITH MINOT TWISS

(WITH PLATES X AND XI)

A survey of the literature on the prothallia of the Filicineae brings out plainly two points: (1) for a number of years those who have studied this side of the life history of ferns have urged that one must not consider the heart-shaped prothallium, whose development was first worked out, the norm from which all variations are comparatively rare exceptions; (2) many investigators have expressed the conviction that gametophytic characters could be used in classification.

BAUKE (**1**) was among the first to urge this latter idea when, in an effort to determine the position of the Cyatheaceae, he searched the gametophyte for decisive characters to add to those afforded by the sporophyte. The examination of the prothallium of this family convinced him that aid could be gained there, and he extended his observations to the Schizaeaceae and meant to take up other families. Before this work was completed, however, he died.

About ten years after this, GOEBEL (**9**) followed a study of the prothallia of a number of epiphytic ferns with a discussion of the light thrown by them on the possible origin of ferns from algae through bryophytes. In his later work (**11**) he adds to his account of different gametophytes and reviews his former conclusions, ending with the statement that "within the single natural groups also one may well recognize a conformity in the formation of the prothallus which is expressed in the possibility of arrranging them in series." From his investigations in 1896 HEIM (**12**) came to similar conclusions as to the value of gametophytic characters.

It certainly seems worth while to review the situation and see whether something may not be added from this side to the points already made by the study of the sporangial and vascular development of the sporophyte. Any such attempt, however, must be preceded by considerable work in two directions: (1) although the

prothallia of most of the genera have been investigated, a review of the literature shows many points untouched, and much, done some years ago without the aid of modern technique, that needs reinvestigation; (2) much experimental work is yet to be done regarding the effect of different conditions upon the morphology of the thallus. As a beginning I have attempted the study of some of the Schizaeaceae.

Historical résumé

The first report on the prothallium of this group was given by KNY (**13**) at a meeting of the Gesellschaft naturforschender Freunde at Berlin in 1868, where he instanced *Aneimia hirta* as forming a cell plate immediately on emergence of the filament from the spore. BURCK (**6**) confirmed this and added that the apical cell when formed was not, as in Polypodiaceae and Cyatheaceae, at the end of the thallus but at one side.

In 1875 BURCK (**7**) made a more extended study of three species of Aneimia (*A. Phyllitidis*, *A. fraxinfolia*, and *A. longifolia*), and laid considerable stress on the fact that the whole filament, by longitudinal wall formation, takes part in the formation of the thallus, while in polypods this is formed only at the end of the filament. He also finds the formation of the further stages of the thallus from a lateral cell and a later initial group and attempts to trace the course of development here more carefully. As an additional character of considerable importance, he gives what he calls the "pousse laterale normale," a lateral wing formed by some prothallia, which often becomes thickly covered with antheridia.

BAUKE in his paper (**2**) points out that this "pousse laterale normale" is only a lobe of the often very irregular male prothallium. The first point made by BURCK he modifies by saying that in the Polypodiaceae and Cyatheaceae longitudinal division does take place in several cells of the filament, but always after the wall has appeared in the terminal cell, while in Aneimia the cells near the spore divide at the same time or even earlier than those at the distal end. The lateral position of apical cell and initials is still insisted upon, though details as given by BURCK are criticized. Neither investigator finds anything to mention as especially characteristic in the development of the archegonia, and both confirm the account of antheridial devel-

opment given by KNY. He (**14**) reported that the first wall in the antheridium is always flat instead of funnel-shaped, as in the polypods, and that the discharge of sperms takes place through a star-shaped break in the cover cell.

When BAUKE (**3, 4**) continued his investigation of different prothallia, he added *Lygodium japonicum* to those of the Schizaeaceae already examined. Here he pointed out the long-continued persistence of the apical cell. He also found on well-developed prothallia archegonia alone at first and antheridia only when the prothallia were considerably older. The exit of sperms here, he reported, is provided for by the throwing off of the cover cell.

HEIM (**12**), working with the same species of Lygodium, confirmed both of these points. He studied also *Aneimia Dregeana*, *A. Phyllitidis*, *A. fraxinfolia*, and *Mohria Caffrorum*, and gives as an additional distinguishing mark of the whole genus warty (*knotige*) thickenings of the side walls of the prothallial cells.

Methods

The soil in which the prothallia used in the present investigation were grown was a mixture of loam and sand. Six-inch pots were filled to within two inches of the top with coarse stones loosely mixed with Sphagnum to facilitate watering and to prevent the dirt from sifting through. One-half an inch of well-packed soil covered this, and over the top a thin layer of soil was sifted through a fine sieve. After sterilization this fineness of the upper soil facilitated the removal of prothallia for study. Pots and soil were sterilized in steam for several hours and, after the sowing of the spores, were kept covered with glass and watered from below. The prothallia were grown in a greenhouse where the temperature was always near 100° F. and lighted with diffuse light from above.

The early stages were obtained free from dirt by sowing the spores on porous clay plates and keeping these in Petri dishes with a little distilled water. Spores were also germinated on the surface of distilled water and of 0.6 per cent. Knopf's solution. Before drawings were made the prothallia were always carefully compared with the same stages on dirt, to determine whether the form or course of cell division had not been altered by the change of medium.

Fungi were kept down by the potassium permanganate solution advised by LANG (**15**). In a letter from Dr. LANG to Dr. COULTER the directions called for a solution decidedly pink; I used with success 0.015^{gr} potassium permanganate in 3.5 liters of water.

Drawings of the early stages were made from living material. The older prothallia were killed with Flemming's weaker solution (**8**), imbedded in paraffin, and the sections stained with safranin and iron alum-hematoxylin.

Lygodium

The species studied was *Lygodium circinatum* (Burm.) Sw. (*L. dichotomum* Sw.), spores of which were obtained through the kindness of Dr. J. N. ROSE, from the botanical gardens at Washington, D. C.

SPORE COATS

The coats reported for the spores of Filicineae are an outer exceedingly delicate epispore, a heavier exine having usually peculiar markings, and a thinner intine which covers the emerging papilla when germination occurs. Paraffin sections of spores of Lygodium, however, showed the possibility of a different situation, and accordingly a study of the development of the coats was undertaken. For this study I was most fortunate in having access to slides prepared by BINFORD (**5**) in his work on the sporangia of *Lygodium circinatum*, and thanks are due him for the aid afforded by his preparations. The slides were stained with safranin and gentian violet, and this should be kept in mind when reading the account of the coloring of the different coats. The sporangia are produced in acropetal succession, so that it is easy to get a clear picture of the different stages.

When the spore mother cell rounds off, the wall is exceedingly delicate, and at the tetrad stage no remnant of it could be identified with any certainty. As *fig. 1* shows, there is a clear space about the tetrad, so that the tapetal protoplasm with its large nuclei does not touch the spores. The protoplasm at the edge of this clear space was carefully examined for traces of the old mother cell wall, but none could be found. Moreover, the same clear space was seen about the spore mother cells themselves in some of the sporangia. Whether the clear space was due to plasmolysis caused by the fixing agent

could not be determined, since there was no opportunity to observe living material.

The wall first formed about the spore is the exine. Very early, before any thickening of this wall has taken place, the color changes from purple to bright red, and the spores increase in size so that the tapetal protoplasm is now close against them. As the exine thickens, a difference in staining becomes apparent, the inner part being red, the outer yellow, but with no clear line of demarkation between them (*fig. 2*, *ex*, *r*, and *y*). No chemical tests could be applied to determine the nature of the substances taking these colors, but from what THOMSON (**16**) says of the colors of megaspore membranes of gymnosperms when stained with safranin, and from what he says of the course of development in these membranes, it seems probable that the red indicated the presence of suberin, and the yellow of pectin.

By this time the intine (*fig. 2*, *i*) has been laid down just within the exine. In the sporangia showing the stage just preceding, the spores were so collapsed that it was impossible to determine whether or not the intine was formed before the exine had differentiated into red and yellow regions.

At a stage shown in *fig. 2* there are here and there in the cytoplasm groups of reddish granules (*fig. 2*, *g*). These are first recognized as very small, deeply staining bodies at the intersections of the protoplasmic network. They increase in size and the protoplasm, either by the breaking down or drawing together of some of the connecting strands, assumes the appearance of a network of coarser mesh, in which, both at the intersections and along the strands, are the now redly staining granules. The nuclei (*fig. 2*, *n*) show the beginning of degeneration in the irregular clumping of the chromatin, and later in the disappearance of the membrane, and it is very likely that the nuclear substance contributes largely to the formation of the granules, which take on a deeper and deeper stain as the nuclei become unrecognizable.

In the older sporangia the granules are larger and larger, of fairly regular shape, as if from the rounding up of viscid matter, but of varying size (*fig. 3*). Some of them are close together, and their position suggests that the larger ones may have come from the running together of two or more of the smaller ones. Those in proximity to the spore

coat adhere to it (*fig. 4*), but for some time not very tightly, so that they are easily pulled away in the cutting. The protoplasm comes to form a delicate continuous sheet between the granules and over their surface (*fig. 5*). The mature wall shows that the protuberances thus formed are still of varying sizes and somewhat irregularly placed, and that they and the exposed position of the exine are covered by the thin layer of cytoplasm (*fig. 6*). In the spores of *Lygodium circinatum* (*figs. 7, 8*), sections in which the coats have been broken apart in the cutting show clearly the delicate intine (*i*), the heavy exine (*ex*) with its two differently staining portions, and the epispore (*e*) of heavy projections formed by the tapetal protoplasm. It is probable that in this epispore we have merely a difference in degree from the more delicate one reported for Filicineae. If the granules were not developed, we should have in the thin sheet of protoplasm covering the spores just such an epispore.

DEVELOPMENT OF THALLUS

The exine of the spores is opaque, so that it was difficult to determine just when chlorophyll was formed. The first definite signs of germination are visible in five to seven days, when the spore coats split and a colorless cell, the first rhizoid, protudes (*fig. 9, a*). The first prothallial cell may be seen as a projecting papilla soon after the rhizoid, but seems to grow more slowly, so that by the time it is well out of the spore the rhizoid is five or six times the longer (*fig. 12*). Very shortly there is seen a smaller papilla pushing from the spore at the side of the first (*fig. 10, p^1*), and as the two protrude farther it becomes clear that the smaller one is a cell cut off from the side of the larger, and that the rhizoid has its origin in turn from this smaller cell (*fig. 12*). The first division of the spore does not separate the rhizoid and first prothallial cell.

The chlorophyll grains are fewer in number and smaller than those in the larger cell, where they are crowded in a dense mass about the nucleus. Sometimes two rhizoids appear, as BURCK reports is always the case in *L. japonicum*, but this seems to happen rarely. Even more seldom the prothallial cell emerges before the rhizoid. The three layers of the spore coat may be distinguished at this stage (*figs. 9, 10*). It is not at all unusual for a spore to produce two fila-

ments (*fig. 11*), and the prothallium then is very like a young prothallium of Hymenophyllum or Trichomanes.

Even before the relation of these three cells is clear, division of the larger prothallial cell by a transverse wall has usually taken place (*fig. 10*). Development now proceeds in one of three ways: (*a*) longitudinal and transverse wall formation may follow so as to produce two rows of nearly equal cells (*figs. 13, 14, 15*); (*b*) an oblique wall may take the place of the first longitudinal wall so that, almost from the first, growth is by an apical cell cutting off segments right and left (*figs. 16, 17*); (*c*) the longitudinal walls may not come in at all, and a filament of a single row of cells is produced (*fig. 18*). The last case seems to be comparatively rare; the others appear about equally often.

This course of development may be altered by varying the conditions in which the prothallia grow. Under a screen of potassium bichromate solution, and in both weak and strong sunlight, filaments of a single row of cells were produced, and these did not broaden to a thallus. The filaments sometimes reached a length of 4 or 5^{mm}, attaining this not through numerous divisions, but by the very unusual length of many of the cells. The small cell between the rhizoid and the first prothallial cell was present in these filaments.

In none of these cultures did antheridia appear, though some of them were kept for a time longer than that within which antheridia are produced under usual conditions. The early appearance of antheridia on filamentous prothallia has been reported as usual. WORONEW (**17**), however, says that he failed to get antheridia on filaments growing in weaker light, but was able to bring about their early production by unfavorable conditions of crowding, drying, etc. In a culture growing in dirt badly overrun by algae, I did find a few prothallia with antheridia produced from the third or fourth cell.

Under a screen of ammoniated copper sulfate, the prothallia, while germinating much later and growing more slowly, broadened in the usual way.

In the ordinary cultures, by the end of ten or twelve days an apical cell is established and a thallus is produced, first spatulate and then, by the more rapid growth of the cells on either side of the apical cell,

heart-shaped (*figs. 19–22*). GOEBEL (**11**, p. 204) has called attention to the fact that for a time the wings of such a prothallium are not of the same size. This unequal lobing is commonly the case with *Lygodium circinatum*, as may be seen from *fig. 22*. The apical cell, however, has clearly a terminal position (*figs. 20–22*), and the inequality of lobing seems in this case to come from the faster growth on one side than on the other. Occasionally the two wings seem to develop equally, and in the end they are of equal size in both cases. As BAUKE reports, the apical cell persists for a relatively long time, but finally gives place to several initials.

SEX ORGANS

Antheridia appear in about three weeks, before the apical cell of the prothallium has been succeeded by the group of initials. Any cell of the prothallium may grow out into a papilla which is cut off to form an antheridium. These usually appear on the lower surface, but are found now and then on the upper surface as well. The first wall of the antheridium is often flat (*fig. 23*), as KNY (**14**) reports, but may be so concave as to touch the basal wall (*fig. 24*). There seems to be nothing here, at least in the ordinary forms, that could be called peculiar to the gametophyte of this family. Occasionally, however, more of a stalk is formed in a manner resembling the antheridia of the Osmundaceae (*fig. 25*). One such case is reported by HEIM (**12**).

The formation of a dome-shaped wall and the cutting off of a cover cell follow, and the central cell divides to form a large number of spermatogenous cells; in Lygodium 128 sperms seem to be the characteristic number.

Within six weeks archegonia have appeared. Examination of a number of prothallia at this date showed some with antheridia only, two with archegonia only, and some with both antheridia and archegonia. (All these prothallia were heart-shaped; crowded prothallia of irregular shape are not included.) Of the first, with antheridia only, there were two forms: (*a*) broadly heart-shaped, with antheridia in great numbers over much of the lower surface and crowded near the initials; and (*b*) younger prothallia, with fewer, more scattered antheridia, and none very near the notch. Of the second kind, I

found but two instances. All prothallia examined at a later stage were of the third kind.

The archegonial cushion is somewhat thicker than that usually figured for polypods, and the archegonia project from it in all directions except from the dorsal surface. The development seems to correspond to that reported for polypods (*figs. 26–28*). There are two neck canal nuclei (*fig. 28*); occasionally four are found, but with no walls (*fig. 29*).

Aneimia

SPORE COATS

The sporangia studied for the development of the spore coat were those of *Aneimia hirsuta* (L.) Sw., in which the spores show parallel ridges set with spines. The material, which had been killed in 70 per cent. alcohol and formalin, was imbedded in paraffin and the sections stained, like those of Lygodium, with safranin and gentian violet.

The earlier stages in the formation of the spore coats agree with those in Lygodium except that the mother cell wall is not so delicate, and traces of it may still be seen after the walls of the spores appear in the tetrad. When the exine begins to show differentiation into red and yellow parts, ridges appear on the outer surface and the red is largely confined to these ridges, only a line of it appearing near the inner edge of the coat (*fig. 30*). Stages were found before the one represented and with barely perceptible ridges, and later ones in which they were more prominent. No trace of granules in the cytoplasm can be seen, though the nuclei have an appearance of degeneration, comparable to that in the same stage of Lygodium. A surface view of the coat at this time shows ridges but none of the spines of the mature coat.

That these spines are built up on the ridges by the activity of the tapetal protoplasm seems evident from stages like that shown in *fig. 31*. The exine shows the red and yellow, but the spines stain purple, and they are very easily pulled away from the ridges. Later these spines change their nature, beginning at the part nearest the ridge, and show the yellow stain except just at the tip.

In the case of *Aneimia hirsuta*, then, the epispore consists of these

spines and a general delicate coat of protoplasm. The exine is a coat of two differently staining portions and with ridges on its outer surface. The intine is first clearly seen at the same stage as in Lygodium, when the exine differentiates into red and yellow portions.

FORMATION OF THE THALLUS

The main work was done with *Aneimia Phyllitidis* (L.) Sw., which Dr. BARNES kindly sent from Mexico at the time of his botanical expedition there in 1908. Younger stages were obtained from spores of *A. hirsuta* from material sent to Dr. CHAMBERLAIN from the Philippine Islands, and from those of *A. Phyllitidis*, which Dr. TRELEASE was kind enough to send from the Missouri Botanical Gardens. Spores were also received from Dr. BRITTON from the New York Botanical Garden, but cultures from these were not successful. For the identification of the specimens I am indebted to Dr. JESSE M. GREEMAN of the Field Museum of Natural History, Chicago.

Germination is somewhat slower than in Lygodium and does not take place till the seventeenth or nineteenth day. The rhizoid is first to protrude and the first prothallial cell follows, but the small cell between them does not emerge as it does in Lygodium. It is only when the filament and rhizoid have attained considerable length that it can be seen at all (*fig. 32*).

The filament does not broaden so early or in so regular a manner as in Lygodium. A spatulate and often irregular thallus is formed (*figs. 34, 35*), and within about ten days initials appear at the side (*fig. 36*). From the rapid division of these initials the thallus takes on the heart-shaped form, the lobes being unequal in size. In this case the larger lobe is the original thallus and the smaller lobe is the younger, as GOEBEL reports for Pteris (**11**, p. 205). In prothallia hitherto studied, this inequality is reported as persistent, but while this was true of *A. hirsuta*, it was not for *A. Phyllitidis*, the lobes of this species finally becoming of equal size, as they do in Lygodium. There is here further reason for agreeing with GOEBEL (**11**, p. 205) when he says, "I do not believe that one can construct a phyletic relationship between apical and lateral position of meristem; . . . . in different sections of the Filicineae both occur."

SEX ORGANS

Antheridia appear in about forty days and the points made in the case of Lygodium may be repeated here: The first wall is *not* always flat; instances are occasionally found of several cells in the stalk; and the sperm number (156) is large. The archegonia appear about ten days later, and have two neck canal nuclei, as do those in Lygodium.

Summary

SPORE COATS

The spores of Aneimia and of Lygodium have three distinct coats. The exine is formed first, and as it broadens its composition changes from cellulose, so that with safranin it stains red, and then red and yellow. The change may be from cellulose to suberin, which stains red, and then to suberin and pectin, as pectin stains yellow. The exine of *Aneimia hirsuta* has ridges on the outer surface.

The intine is the second coat formed, and is the one which covers the filament when it emerges from the spore. It remains a delicate cellulose wall.

The epispore is the last to form, and in both Aneimia and Lygodium is produced by the activity of the tapetal protoplasm. In Lygodium granules appear in the protoplasm, increase in size, and adhere to the exine. In *Aneimia hirsuta* spines are formed on the ridges of the exine. The protoplasm forms a thin sheet over these projections and over the intervening surface of the exine.

DEVELOPMENT OF THE THALLUS

The first wall of the spore does not separate the rhizoid and first prothallial cell, but the spore contents divide into two cells of unequal size, from the smaller of which the rhizoid is produced.

The apical cell of Lygodium is terminal, appears early, and is remarkably persistent. In Aneimia it appears later and is lateral.

The lobes of the heart-shaped thallus are at first unequal. In *Aneimia hirsuta* this inequality is permanent, but in *Aneimia Phyllitidis* and in Lygodium the lobes become later of the same size.

SEX ORGANS

The general course of development in both antheridia and archegonia does not differ from that in the Polypodiaceae.

The first wall of the antheridium is not, as reported, always flat, but may be so concave as to touch the basal wall.

The number of sperms is large.

The archegonia have two neck canal nuclei.

Conclusions

There is nothing in the formation of the antheridium or in the unequal lobing of the prothallium that can be considered characteristic of the genera.

The fact that the rhizoid is not produced as a result of the first division of the spore has not yet been reported for other Filicales, and may possibly be peculiar to the Schizaeaceae.

The large number of sperms produced, the occasional stalk of the antheridium, and the frequent occurrence of two prothallial filaments from the spore, are characters which would place the Schizaeaceae with the more primitive families of the Filicineae.

I wish to express my thanks to Dr. JOHN M. COULTER, at whose suggestion this work was undertaken, and to Dr. CHARLES J. CHAMBERLAIN for his kind direction and criticism.

THE UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES X AND XI

Reference letters: *a*, rhizoid; *e*, epispore; *ex*, exine; *g*, granules which form epispore; *i*, intine; *n*, nucleus; *p*, *p'*, prothallial cells; *r*, red portion of exine; *y*, yellow portion of exine.

PLATE X

Lygodium circinatum

FIG. 1.—Tetrad. ×1050.

FIG. 2.—Portion of spore wall and tapetal protoplasm with granules and nucleus. ×1050.

FIG. 3.—Later stage in the formation of granules. ×1050.

FIG. 4.—Portion of exine with granules adhering to form the epispore. ×1050.

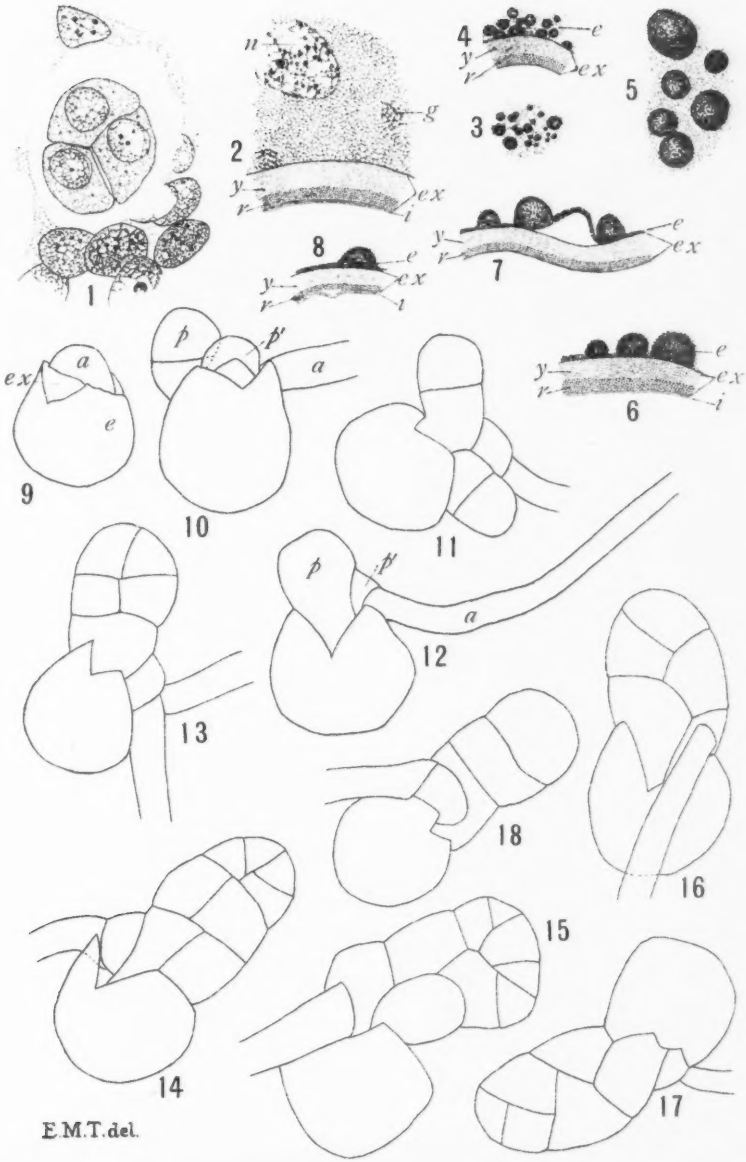
FIG. 5.—Appearance of granules and tapetal protoplasm when spores are mature, showing irregular size and distribution of granules. ×1050.

FIG. 6.—Portion of wall of mature spore, showing epispore with projections of varying size and irregular distribution. ×1050.

FIG. 7.—Portion of spore wall, showing epispore pulled away from the exine; but the view is oblique so that the epispore appears wider than it really is. ×1050.

FIG. 8.—Portion of spore wall with intine pulled away. ×1050.

FIG. 9.—Germination of spore showing rhizoid emerging. ×300.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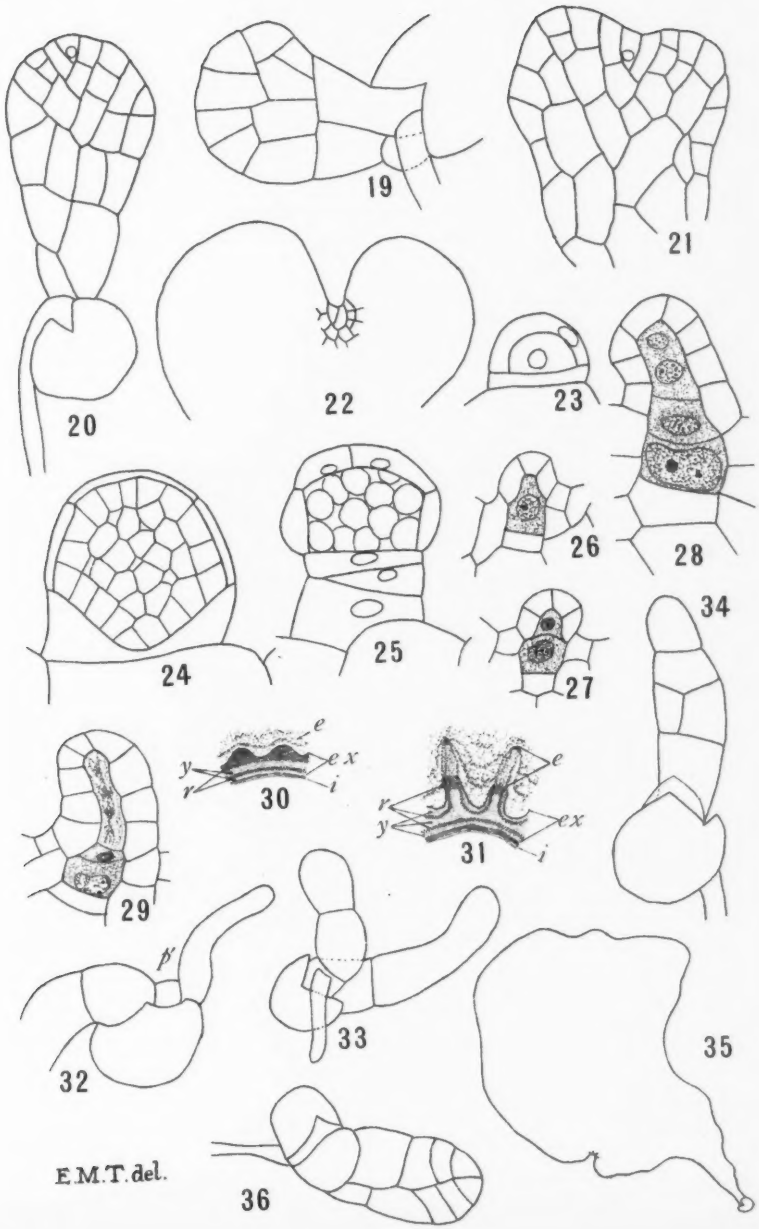
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FIG. 10.—Later stage, showing two papillae (prothallial cells). ×300.

FIG. 11.—Two prothallial filaments coming from one spore. ×300.

FIG. 12.—Further stage, showing relation of prothallial cells and rhizoid. ×300.

FIGS. 13–15.—One method of development of thallus. ×300.

FIGS. 16, 17.—Same stages in thallus developed in another way. ×300.

FIG. 18.—Third way of developing the thallus. ×300.

PLATE XI

Lygodium circinatum

FIGS. 19–22.—Later stages in development of thallus, showing terminal position of apical cell, and in *fig. 22* the unequal size of the lobes. *Fig. 19*, ×300; *figs. 20, 21*, ×200; *fig. 22*, ×50.

FIG. 23.—Young antheridium with first wall flat. ×700.

FIG. 24.—Antheridium at mother cell stage, with first wall concave. ×700.

FIG. 25.—Antheridium with stalk. ×700.

FIGS. 26–28.—Development of archegonium. ×300.

FIG. 29.—Archegonium with remnants of four neck canal nuclei. ×300.

Aneimia hirsuta

FIG. 30.—Portion of spore wall. ×1050.

FIG. 31.—Later stage of same. ×1050.

FIG. 32.—Germination of spore. ×300.

FIG. 33.—Two prothallial filaments from one spore. ×200.

FIG. 34.—Development of thallus. ×300.

FIG. 36.—Still later stage showing lateral position of initials. ×200.

Aneimia Phyllitidis

FIG. 35.—Later stages of development of thallus. ×50.

THE STRUCTURE AND RELATIONSHIP OF URNULA GEASTER

F. D. HEALD AND F. A. WOLF

(WITH PLATE XII AND THREE FIGURES)

During the season of 1909 the writers found an interesting ascomycete growing abundantly in the vicinity of Austin, Texas. On attempting to identify the species it was traced to *Urnula geaster* Peck,[1] which was collected at Austin, in 1893, by Dr. L. M. UNDERWOOD and sent to Mr. C. H. PECK for identification, but the characters observed did not agree with PECK's description in several important points. For this reason a package of fresh specimens was sent to Mr. PECK, who replied that they were the same as the original *Urnula geaster* which he had previously described, but that his description was lacking in a number of important points, since only dried specimens had been available for making the original diagnosis. This description has been corrected by Mr. PECK[2] in his recent report, in accordance with the examination of fresh specimens sent to him by the authors.

Since this fungus has recently been made the basis of a new genus, *Chorioactis*,[3] and the material used for the study consisted only of dried herbarium specimens, the careful study of its anatomy was undertaken to determine the validity of its separation from Urnula.

Habitat

The ascomata of *Urnula geaster* are found growing, either singly or in groups, from roots and portions of stumps of the small-leaved elm (*Ulmus crassifolia*) which are old and somewhat disintegrated. They may be clustered close to the base of the stump, or they may originate from the roots at some point distant from it. In all cases the stipe or stalk of the apothecium comes from a point 5–10^{cm} below the surface of the ground.

[1] SACCARDO, P. A., Sylloge Fungorum **11**:422. 1895.

[2] PECK, C. H., Report of N. Y. State Botanist for 1908. pp. 31, 32. 1909.

[3] KUPFER, E. M., Bull. Torr. Bot. Club **29**:142. 1902.

According to the original description, the fungus was recorded as growing on the "ground"[4] (ad terram).[5] The writers have examined a large number of specimens in the field, and in every case a direct connection with subterranean roots could be established. The ease with which the stipe breaks from its point of attachment and also the depth of the roots in the soil may have been the cause of the original error in observation.

Structure

The brown septate hyphae grow over the surface of the decaying wood as a loose network, or become aggregated into strands or compact layers in intimate contact with the surface of the root.

Specimens of the apothecia have been collected in the cooler months, from October to April. Their appearance only during this portion of the year is undoubtedly due to the favorable conditions of moisture and temperature. The apothecia can generally be found in abundance following a short rainy season during the period mentioned. They begin their development, however, in May or June and grow slowly through the dry summer period, reaching maturity in the months mentioned above. This last observation was contributed by Professor W. H. Long, Amarillo, Texas, and has been confirmed by the writers.

The mature apothecium while still closed is rather thick club-shaped (*text fig. 1*), with a stalk or stipe shorter than the apothecial cavity or equaling it in length. While this is the typical form, specimens which originate from deep-lying roots may have much longer stipes; others growing from more superficial roots may be nearly sessile and rather globular in form (*text fig. 2*). The apothecia before dehiscence vary in diameter from 1.2 to 3.5^{cm} in the broadest portion, and in length from 4 to 12^{cm}. The stalk varies from 0.75 to 1.5^{cm} in diameter and is 1–5^{cm} in length. The outer surface of the entire fructification is covered with a dense chocolate-brown tomentum. The cut surface of the stalk and wall is pure white, while the hymenial layer is yellowish white (28),[6] becoming with age light leather colored (8).[6] The fresh apothecia are of a soft leathery texture, becoming firmer with desiccation.

[4] Peck, C. H., Report of N. Y. State Botanist **46**:39. 1893.

[5] Saccardo, P. A., *l. c.* 422.

[6] *Ibid.*, Chromotaxia. 1894.

As soon as the apothecium reaches maturity, the wall begins to show several longitudinal fissures, which ultimately separate the hymenial portion into 4–6 segments or rays (*text fig. 2*). These rays begin to curve outward as soon as they become separated at the tips, and may come to stand at right angles to the stipe (*text fig. 3*). This position of the rays gives the open apothecium a Geaster-like appearance. In the case of deep-seated apothecia, the rays do not become

FIG. 1.—A series of ascomata of *Urnula geaster;* two of them bisected to show the extent of the apothecial cavity and the stipe.

completely recurved, since their movement is partially prevented by contact with the surrounding soil.

Soon after the dehiscence of the apothecium, dustlike clouds of spores may be seen to rise in puffs from the exposed hymenial surface. This characteristic expulsion of the spores, together with the form and color of the fruiting structure, has secured for the fungus the popular name of the "devil's cigar." A somewhat similar expulsion of spores has been noted in various species of Peziza, Helvella, and Bulgaria.[7] The puffing of the spores is apparently due to loss of moisture, since mature open specimens show the characteristic puffing when removed from a damp chamber to air of the room. While this is probably

[7] MASSEE, G., Textbook of Fungi 110. 1906.

the normal cause of the expulsion of the spores, the same phenomenon may be induced by the release of tension due to breaking the rays. After the spores have been expelled, the release of the tension in the hymenial surface causes the segments of the apothecium to be raised and curled inward (*text fig. 2*).

FIG. 2.—Apothecia of various forms, showing origin from roots and the manner of dehiscence.

The wall of the apothecium is thick, reaching 3.5^{mm} in many cases. The original description does not specify the thickness, while KUPFER[8] gives 2.5^{mm} as the extreme measurement. This difference may be explained easily by the fact that only dry material was available in all the previous studies.

The wall of the apothecium shows in section a region of brown hairs, about 0.1^{mm} in thickness, which is the cause of the brown tomentose exterior. Just below the hairy layer is a relatively narrow cortical zone made up of densely interwoven hyphae, while the remain-

[8] KUPFER, E. M., Bull. Torr. Bot. Club **29**:143. 1902.

der of the wall consists of a loosely interwoven network of filaments which become denser and more closely aggregated in the hypothecial region. The wall, including cortical portion, medullary portion, and hypothecium, may be 2.5^{mm} in thickness, and the hymenium may be 0.75–0.85^{mm} in thickness. It should be noted in this connection that the apothecial wall is composed of distinct hyphae, and that they are never aggregated to form a pseudoparenchyma. This fact is directly opposed to the observations recorded by KUPFER,[8] stating

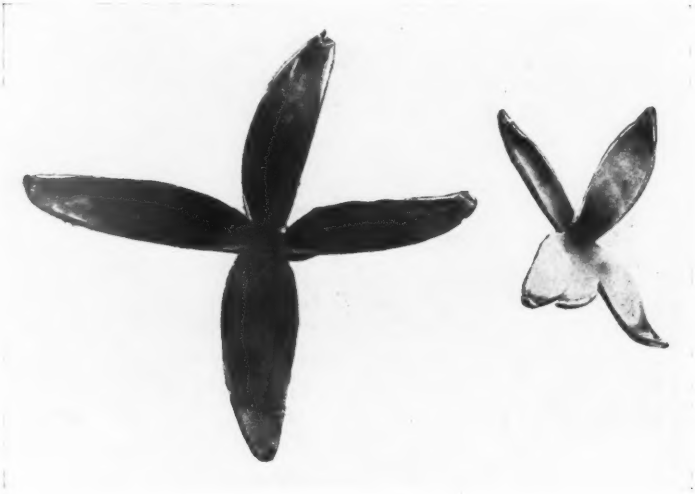

FIG. 3.—Apothecia viewed from above, showing the Geaster-like appearance.

that "the tissue is made up wholly of large parenchymatous cells." She even gives the measurements of these cells and they are also illustrated in the plate accompanying the paper. The size of these so-called parenchymatous cells agrees practically with our measurements of the intercellular spaces in the immature specimens as shown in *fig.* 7.

The erroneous observation mentioned above is without doubt due to the fact that the dry specimens, which were the only ones used, were not completely mature. Previous to the dehiscence of the apothecium the hyphae which make up the wall are very small in

diameter, being about 1.5 μ. *Fig. 7*, which was drawn from fixed (chromacetic acid) and imbedded material from young specimens, might easily have been interpreted as representing a pseudoparenchyma. After the dehiscence of the apothecium, the hyphae constituting the wall enlarge rapidly to 3–6 times their diameter in young specimens, reaching a thickness of 4.5–9 μ (*fig. 8*). At this stage it would seem almost impossible for even the most careless observer to interpret the structure as a pseudoparenchyma, since the hyphae are so large that even in dried specimens they could not be mistaken for cell walls. Even in the most compact part of the subhymenium the hyphae and intercellular spaces are easily distinguished (*fig. 4*).

The asci are 700–800 μ in length and 14–17.25 μ in diameter. This is somewhat in excess of the measurements given for *Urnula craterium*,[9] the type specimen of the genus. The ascus is nearly uniform in diameter and shows a short characteristically curved basal portion. This character and the origin of the asci from the subhymenium is shown in *fig. 4*. The free end of the ascus is bluntly rounded and shows an apical pore which permits the rupture of the wall when the spores are expelled. The eight continuous hyaline spores are confined to the upper two-thirds of the ascus, and are arranged with their tips slightly overlapping, but in a single series. They are oblong-fusiform and distinctly flattened on one side, 54–68 μ long by 10–13 μ wide; each spore contains 3–5 prominent guttulae (*fig. 5*). Numerous branched septate paraphyses are present, which are uniform in diameter throughout (slightly less than 2 μ) and do not show a terminal enlargement as figured by KUPFER (*l. c. pl. 8. fig. 4*).

Systematic position and relationship

As a result of a comparative study of several species of Urnula and Geopyxis, KUPFER (*l. c.* 142) has made *Urnula geaster* Peck the type of the new genus Chorioactis. The basis for the separation is stated as follows: "That it is not an Urnula seems to me just as evident from its external appearance as from an examination of its tissues. A comparison of internal characters shows, however, that there is no possible relation with *Urnula craterium*. The tissue is

[9] SACCARDO, P. A., Sylloge Fungorum **8**:549. 1890.

made up wholly of large parenchymatous cells, those of the excipulum averaging 34 μ in diameter, those of the hypothecium 10–14 μ." In our study of the structure of *Urnula geaster* we have shown, by detailed examination of material fixed in chromacetic acid and stained with iron alum-hematoxylin, that the wall of the apothecium is not parenchymatous, but composed of distinct interlacing hyphae with prominent intercellular spaces. The supposed parenchymatous wall was the main character which separated *Urnula geaster* from *Urnula craterium*, the type of the genus. Since this supposed character was based entirely upon erroneous interpretation of the structure, there can be little ground for the establishment of a new genus. A comparison of some of the characters of *Urnula geaster* and *Urnula craterium* will indicate still further that there is little ground for this separation.

	Urnula craterium (Schw.) Fries	*Urnula geaster* Peck
Size	$3–7 \cdot 5 \times 7^{cm}$	$1 \cdot 2–3 \cdot 5 \times 4–12^{cm}$
Asci	$400–500 \times 14–15\ \mu$	$700–800 \times 14–17 \cdot 25\ \mu$
Spores	$23 \cdot 33 \times 8–13\ \mu$	$54–68 \times 10–13\ \mu$
Consistency	Leathery	Leathery
Wall of apothecium	Interlacing hyphae	Interlacing hyphae

The agreement of our specimens with *Urnula craterium*, the type of the genus as established by FRIES, makes impossible any separation of the species under discussion from the genus Urnula. Therefore the original name of *Urnula geaster*, as given by PECK, should be retained.

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EXPLANATION OF PLATE XII

FIG. 1.—Portion of cross-section of the apothecium, showing the different layers. $\times 15$.

FIG. 2.—A single ascus with branched paraphysis. $\times 83$.

FIG. 3.—A single ascus, showing the position of spores. $\times 330$.

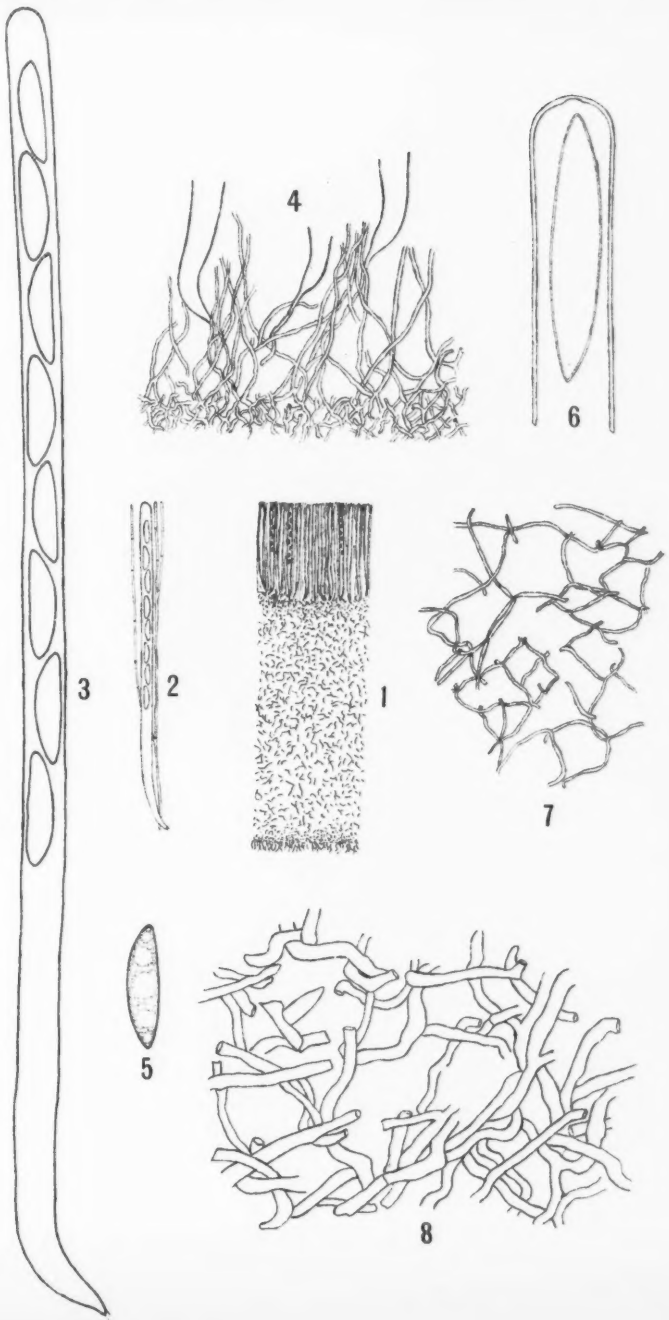
FIG. 4.—Origin of paraphyses and asci from the subhymenium. $\times 330$.

FIG. 5.—A single spore showing empty spaces from which the reserve food in the form of oil has been dissolved. $\times 330$.

FIG. 6.—Tip of ascus showing apical pore. $\times 660$.

FIG. 7.—Interlacing hyphae from the wall of the apothecium preceding dehiscence. $\times 330$.

FIG. 8.—Interlacing hyphae from the wall of a mature open apothecium. $\times 330$.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THE EXCHANGE OF MATERIAL BETWEEN NUCLEUS AND CYTOPLASM IN PEPEROMIA SINTENISII[1]

WILLIAM H. BROWN

(WITH PLATE XIII)

In describing the development of the embryo sac of *Peperomia sintenisii*, the writer showed that at the time of the fusion of the sexual nuclei globules of cytoplasm were taken into the fusion nucleus. This phenomenon was not studied in detail at that time, but seemed to be worthy of further study, as it offered the possibility of throwing some light on the relation between cytoplasm and nucleoplasm. For the investigation of this problem material which had been fixed in chromacetic acid was cut 10 μ thick and stained with Flemming's triple or Haidenhain's iron alum-hematoxylin and eosin.

As the male nucleus of *Peperomia sintenisii* approaches the egg, just before fusion, both nuclei come to have cup-shaped depressions in the sides facing each other (*figs. 1–5*); the other parts of the nuclei, however, have a fairly regular shape. The depressions in the two nuclei may correspond closely to each other (*figs. 2, 3*), or they may not (*figs. 1, 5*). The number of depressions is also variable in different nuclei, there being one in each nucleus in *figs. 1, 2*, two in *fig. 3*, while *figs. 4* and *5*, which were taken from the same pair of nuclei, show three depressions in each nucleus. When the two nuclei come together, the nuclear membranes disappear where the nuclei touch each other, but remain intact around the depressions, the cytoplasmic contents of which appear as vesicles within the nucleus (*figs. 6–18*). *Fig. 6* shows a case in which one vesicle is caught in the nucleus while two others are probably in the process of being inclosed. *Fig. 7* shows one vesicle inclosed in the nucleus and another nearly inclosed. The number and size of the vesicles, like the depressions giving rise to them, are variable (*figs. 8–18*). When the cytoplasm is first inclosed in the vesicle, it takes the usual cytoplasmic stains (*figs. 7–9*), but it gradually stains less and less until the vesicles are distinguishable from the clear nuclear sap only by the membranes which

[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 11.

surround them. *Figs. 10–18* show various stages in the disappearance of the staining reaction in the cytoplasmic vesicles. In *figs. 10* and *11* there is a trace of stain; in *figs. 12–14* there is no stain in one of the vesicles; while *figs. 15–18* show vesicles the contents of which are indistinguishable from the surrounding clear nuclear sap. Shortly after this stage the membranes around the vesicles begin to break down and disappear (*figs. 19, 20*).

The phenomenon just described seems to be a constant characteristic of fertilization in *Peperomia sintenisii*, as no fusions were observed in which cytoplasm was not taken into the nucleus, although more than 50 nuclear fusions of this stage were studied. That the phenomenon is not due to shrinkage in fixing is shown by the regular appearance of the other nuclei in the sac, as well as of all parts of the sexual nuclei except the sides facing each other at the time of fusion. This view is confirmed by the further development, when by focusing the vesicles can be seen to be completely inclosed within the nucleus. This is shown in *figs. 12, 13*, and *15*, where one nucleolus is below and the other above the vesicle.

The growth of any nucleus must be at the expense of material obtained from the cytoplasm, and so it is evident that some material must pass from the cytoplasm to the nucleus. The phenomenon just described, where a vesicle of cytoplasm becomes nucleoplasm, seems to show that all of the essential constituents of cytoplasm may be changed into nucleoplasm.

The presence of the cytoplasmic vesicle within the nucleus gives the nucleus a greater surface for the intake of cytoplasm and this may be of advantage to it at this stage.

The membrane around the cytoplasmic vesicle did not disappear, however, before the cytoplasm seemed to be changed to nucleoplasm. This supports LAWSON'S conclusion that the nuclear membrane is formed by the cytoplasm coming into contact with the nuclear sap, for the membrane here as in other fusing nuclei disappears, probably by absorption, when nucleoplasm is on both sides of it. LAWSON bases his conclusion on the fact that in the reorganization of nuclei the nuclear membrane appears at the time that the clear nuclear sap appears outside the chromosomes and in contact with the cytoplasm. The same thing appears to be true in the case of *Peperomia sintenisii*

(BROWN **'08**). It is not necessary to assume that the process is a simple chemical precipitation, because it may be a physiological response.

The question now arises as to what happens when the nuclear membrane disappears during prophase. In *Peperomia sintenisii* the incipient spindle fibers form a dense felt over the nucleus before the membrane disappears. The appearance of spindle fibers before the disappearance of the nuclear membrane has been described in Lilium, Podophyllum, and Helleborus (MOTTIER **'97**), Equisetum (OSTERHOUT **'97**), Cobea and Gladiolus (LAWSON **'98, '00**), Pellia and Anthoceros (DAVIS **'99, '01**), Lavatera (BYXBEE **'09**), Osmunda (SMITH **'00**), Larix (ALLEN **'03**), and a number of other genera. In *Peperomia sintenisii*, as in a number of other forms, the appearance of the cytoplasm around the nucleus is at this stage quite different from what it was before the appearance of the fibers, and the disappearance of the membrane may be connected with this change of the cytoplasm.

When the cytoplasmic vesicle in *Peperomia sintenisii* becomes nucleoplasm, the question arises as to whether the cytoplasm has passed into the nucleus and nucleoplasm into the vesicle, or whether the cytoplasm is changed while in the vesicle. If, as seems probable, the nuclear membrane is formed by the interaction of the nuclear sap and the cytoplasm or some part of the cytoplasm, it seems evident that that part of the cytoplasm which participates in the formation of the membrane could not pass through the membrane, because, if it did, on coming into contact with the nucleoplasm it would only thicken the membrane. This does not mean, however, that no substances can pass from the nucleus to the cytoplasm or from the cytoplasm to the nucleus. It seems evident, however, that in *Peperomia sintenisii* the cytoplasmic vesicle, on account of its position, is converted into nucleoplasm through the activity of the nucleus, for there is no more reason for thinking that this part of the cytoplasm would change itself than there is for thinking that all of the cytoplasm would do the same thing. Then since the cytoplasm cannot pass unchanged into the nucleus, it is probable that some substance passes out into the cytoplasm and changes the cytoplasm in such a way that it may then be taken into the nucleus. It is not likely that

this method of exchange is confined to this particular case, but since all of the essential constituents of protoplasm go into the nucleus of *Peperomia sintenisii*, it seems probable that the same thing happens in other organisms, and that this is rendered possible, as is probably the case in *P. sintenisii*, by the action of some substance or substances which continually pass out of the nucleus and cause a change in the cytoplasm.

It may be that the previously mentioned change of the cytoplasm around the nucleus, just before division, is connected with the accumulation, outside the nucleus, of the substance which has passed from the nucleus; and that the disappearance of the nuclear membrane is connected with a change of the cytoplasm causing a suspension of the reaction between the cytoplasm and nucleoplasm. The disappearance of the membrane might then be by absorption, analogous to the absorption of the nuclear membrane between two fusing nuclei. The substances which made possible this absorption, by accumulating outside the nucleus, would probably not be the same as those which, by reacting with the cytoplasm, caused the formation of the nuclear membrane. On the other hand, it seems not improbable that during division the nuclear sap loses its power of reacting with the cytoplasm, for it may become mixed with the cytoplasm or form a definite part of the cytoplasm, as in the maturation of certain animal eggs.

At the division of a cell of *Peperomia sintenisii*, as in other plants, the nuclear sap and nucleolus are lost in the cytoplasm; and so it is evident that nucleoplasm may also become a part of the cytoplasm. In many animals the clear substance which passes from the nucleus at maturation goes to form a special part of the embryo, and the passing of nucleoplasm to the cytoplasm has been considered as a method by which the chromosomes influence the development and activities of an organism. The observations on *Peperomia sintenisii* seem to show that some substance or substances are being constantly passed from the nucleus to the cytoplasm, and this may also be a means by which the nucleus controls the activities of the cell. The control of the cytoplasm by the "resting" nucleus seems to be well illustrated in the formation of the blepharoplast of Derbesia (DAVIS '08). Here radiations pass from the nucleus out to the cell wall, and at the ends of these radiations granules which fuse to form the blepharoplast are laid down.

Summary

At fertilization in *Peperomia sintenisii* globules of cytoplasm surrounded by a nuclear membrane are inclosed in the fusion nucleus. The cytoplasm globule gradually assumes the appearance of nuclear sap, after which the membrane around it disappears.

This phenomenon seems to show that all of the essential constituents of cytoplasm can be changed to nucleoplasm.

The appearance of a nuclear membrane when cytoplasm and nuclear sap meet, and the absorption of the membrane when nucleoplasm is on both sides of it, seem to show that the nuclear membrane is formed by the interaction of cytoplasm and nuclear sap.

If the membrane is formed in the above manner, it is probable that the contents of the cytoplasmic vesicle could not be changed to nucleoplasm by a gradual exchange of material between the nucleus and cytoplasm, for all of the constituents of the cytoplasm could not pass through the membrane, because some would react with the nucleoplasm and thicken the membrane. Since, however, the cytoplasmic contents of the vesicle are changed, this change is probably due to some substance which has come from the nucleus.

It is probable then that cytoplasm before being taken into a nucleus is changed by some substance which has passed from the nucleus, because it is not likely that *Peperomia sintenisii* differs from other organisms in such a fundamental activity of protoplasm.

The passage of substance from the nucleus is probably continual and may be a means by which the "resting" nucleus controls the cytoplasm.

The material, which made these observations possible, was collected in Jamaica by Professor D. S. JOHNSON, to whom the writer is greatly indebted.

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EXPLANATION OF PLATE XIII

All drawings were made with the aid of a camera lucida, using a No. 8 ocular and a 1.5^{mm} objective.

FIG. 1.—Male and female nuclei of *Peperomia sintenisii* approaching each other; each nucleus shows one depression in side facing the other.

FIG. 2.—Later stage of same.

FIG. 3.—Male and female nuclei in contact; each shows two depressions.

FIGS. 4, 5.—Two sections through same pair of nuclei, each of which contains three depressions.

FIG. 6.—Early stage of fusion; nuclear membrane has disappeared where nuclei have come into contact with each other, causing the entire inclosure of one and partial inclosure of two other cytoplasmic globules.

FIG. 7.—About same stage as *fig. 6;* one globule entirely, another partially inclosed.

FIG. 8.—Fusion nucleus containing one vesicle of cytoplasm surrounded by a nuclear membrane.

FIG. 9.—Fusion nucleus containing two vesicles of cytoplasm.

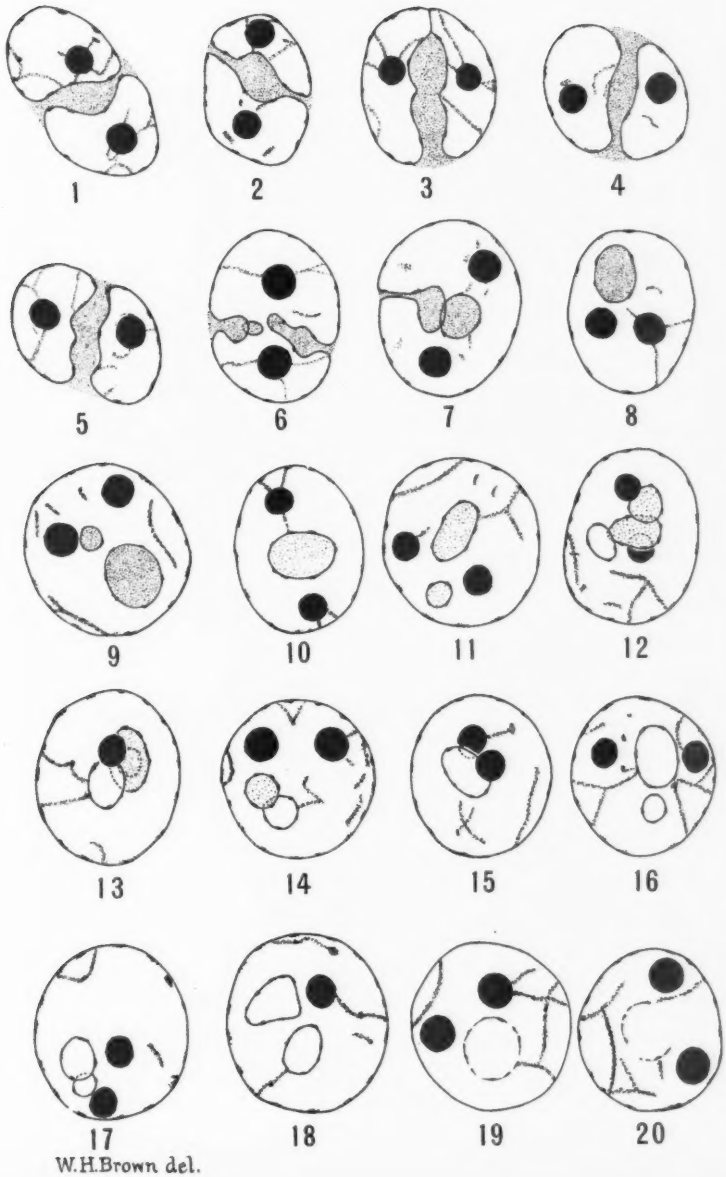
FIGS. 10, 11.—Later stages; the cytoplasm stains less densely.

FIGS. 12–14.—Still later stage; the staining reaction of cytoplasm has completely disappeared from one of the vesicles.

FIGS. 15–18.—The contents of the vesicles are indistinguishable from the surrounding clear nuclear sap.

FIG. 19.—The membrane around the vesicle is beginning to disappear.

FIG. 20.—Later stage in the disappearance of the membrane around the vesicle.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W.H.Brown del.

DEVELOPMENT OF THE HELVELLINEAE

I. HELVELLA ELASTICA

W. A. McCUBBIN

(WITH PLATES XIV–XVI AND ONE FIGURE)

Our knowledge of the developmental stages of the Helvellineae has been restricted to the Geoglossaceae, and is mainly due to the researches of DITTRICH (**9**) on *Leotia gelatinosa* and *Mitrula phalloides*. Practically nothing is known of the ontogeny of the Helvellaceae and Rhizinaceae. DITTRICH's investigations on the Geoglossaceae showed, contrary to the opinion formerly held, that these forms, in their young stages at least, were unmistakably angiocarpous. He discovered that the young fruiting body of Leotia was surrounded by an envelope of hyphal filaments, which later gelatinized, and was then disrupted by the expanding ascoma beneath. In *Mitrula phalloides* the phenomena were quite similar, but among the Helvellaceae which he examined there were no stages young enough to enable him to decide as to the presence or absence of an envelope. In Mitrula the ascogenous hyphae were found to originate from hyphae which were differentiated in the central part of the fruiting body. These hyphae are long, stain readily, and are closely massed, while their nuclei are large, with a prominent nucleolus and a thin chromatin thread. From these filaments the ascogenous hyphae grow toward the hymenial layer, where a rapid branching takes place, succeeded by the formation of asci.

DURAND (**10**), in his admirable monograph on the Geoglossaceae, makes reference to DITTRICH's investigations on the envelope in Leotia and Mitrula, and remarks, "observations of my own on several different species, before I learned of DITTRICH's work, point unmistakably to the same conclusion." DURAND states that he found a veil in *Mitrula phalloides*, *Microglossum viride*, *Cudonia lutea*, and *Spathularia velutipes*, but "sections of quite young ascomata of *Geoglossum glabrum*, *Gleoglossum difforme*, and *Trichoglossum velutipes* have shown no traces of such a membrane." Finally he affirms the belief that "when the development of the Discomycetes shall be

better understood, it will be found that in none of them, not even in the Helvellineae, is the hymenium exposed from the first."

An obstacle to the ready solution of this and other details of ontogeny is, as DITTRICH has already pointed out, the difficulty of obtaining young stages either by collection or culture. BREFELD (**3**) made attempts to cultivate the spores of several Helvellineae, but though a mycelium was produced in some cases, no fruiting bodies could be obtained, and often germination did not take place at all. MOLLIARD (**16**) had better success with spores of *Morchella esculenta*, from which he was able to procure a vigorous mycelial growth. This mycelium produced conidia identical with those of Costantinella, and when planted in a compost of apples gave rise to a couple of small fruiting bodies of the morel.

In regard to the taxonomy of the Helvellineae various criteria have been adopted. SCHROETER'S (**17**) classification bases the distinction between this group and the Pezizineae on the supposed gymnocarpous origin of the fruiting body in the former and the angiocarpous in the latter. BOUDIER (**2**), in endeavoring to construct a natural classification of the Discomycetes, adopts a feature other than angiocarpy or gymnocarpy as a basis. He looks upon the Helvellineae as a polyphyletic group: one part, comprising the Helvellaceae and the Rhizinaceae, being placed in that division of the Discomycetes (Operculés) whose asci open by an apical cap; and the other, consisting of the Geoglossaceae, being relegated to a second division (Inoperculés) whose asci open merely by a pore. BREFELD found on examination of several Discomycetes that when an ascogonium is present the ascogenous hyphae appear quite early, but when the fertile filaments spring from ordinary hyphae, as he stated to be the case in several of the Helvellineae and some Pezizas, the ascogenous hyphae are differentiated at a much later stage. He thought that as the ascogonium occurs in simple forms but not in the more specialized, and that as the specialized forms themselves differed, according to the species, in the time at which the ascogenous hyphae appeared, the gradually later and later differentiation of the fertile filaments would form a very good basis for the arrangement of the Discomycetes in a natural series, at the end of which the Helvellineae would probably stand.

The following investigations have been undertaken for the purpose of enlarging our knowledge of the ontogeny of the Helvellineae, and in the hope that fuller information will be of assistance in dealing with the phylogeny of the group. The complex fructifications of the Helvellineae could scarcely be expected to furnish promising material for working out some of the cytological problems that have been attracting attention in certain other divisions of the Ascomycetes, involving the sexuality of the Ascomycetes and their affinities with other Thallophyta, yet certain phenomena have come to light that prove interesting and suggestive.

Rather complete series of several genera of the Helvellineae have been accumulated and are being studied. Any theoretical considerations will be deferred to a later paper.

Helvella elastica

MATERIAL AND METHODS.—The young stages of the material examined were taken from a bank of loose damp sand in High Park, Toronto, and were collected in July 1908. They were fixed in several ways and cut in series in paraffin. Staining was tried with Erlich's hematoxylin, iron hematoxylin, safranin, eosin, rubin S, Flemming's triple stain, methylene blue, and several others. Iron hematoxylin and Flemming's triple stain gave the best results, the former making the nuclei prominent and the latter bringing out the general features. Macerations were also found to be valuable, the crushed material being stained on the slide with safranin or methylene blue.

Some tentative attempts were made to produce the mycelium of several Helvellineae from spores, with the hope of arriving at the young stages of the fruiting bodies by laboratory cultures. The results, however, are as yet insufficient to justify any statement.

I would here acknowledge my indebtedness to Professor J. H. FAULL for material and advice throughout this investigation, and to Mr. R. B. THOMSON for many courtesies.

MYCELIUM.—The mycelium of *Helvella elastica* is easily obtained by washing the earth surrounding young fruiting bodies. It consists of long, sparsely branched, interwoven filaments about 0.03–0.04^{mm} in diameter. These filaments are often connected by anastomoses (*figs. 3–5*). The cells are multinucleate, containing 2–16

or more small densely staining nuclei. These are frequently arranged in pairs (*figs. 3, 4*). Often one finds a pair separated by a septum, a condition of affairs similar to this being described by STOPPEL (**21**) for *Eremascus fertilis*. Metachromosomes such as CLAUSSEN (**7**) describes for Boudiera are also found here. Two or more are visible lying parallel to the cross walls of the thread, and appear to be more conspicuous near the end of a filament than in its older parts.

THE ASCOMA.—Here and there a specially vigorous growth of the mycelium produces knots or clumps (the fruiting bodies), the cells of which are shorter, thicker, and more branched than those of the surrounding filaments. The earliest stages of the fruiting bodies procured were about 0.5^{mm} in diameter. At this stage the young Helvella consists of a short thick stem spreading out insensibly into the mycelial growth of the substratum, and surmounted by a round bulbous cap of slightly larger diameter than the stem (*fig. 6*). There is at this period no indication of fertile filaments. A membrane and a palisade layer inclose the whole fruiting body, but the palisade hyphae are not yet well differentiated. In stage 2 (diam. 1^{mm}, *fig. 7*) storage cells appear, the palisade layer shows more regularity, while the membrane has been much broken by the rapid growth. Stage 3 (diam. 1.25^{mm}, *fig. 8*) shows the beginning of paraphyses on the site of the hymenium, an increased number of the storage organs, and the presence of a few ascogenous hyphae. The membrane is now represented merely by a few flakes. The hymenium does not grow rapidly at first, however, being outstripped in this respect by the stem and sterile part of the cap. As a result the hymenial surface may assume a flat or even concave shape, as shown in stage 4 (*figs. 9, 66*). Here the membrane has almost disappeared, only a few degenerate threads or masses of cells being distinguishable. The paraphyses are now numerous and the ascogenous hyphae are beginning to branch or even to form hooks. In *fig. 10* the fruiting body has assumed the characteristic saddle shape of the Helvellas. There has been from stage 4 a rapid enlargement of the hymenial surface, which takes place, however, as DITTRICH has pointed out, with considerable irregularity, thus accounting for the peculiar shape of the adult ascoma.

THE PALISADE LAYER.—Surrounding the entire fructification in

early stages, and persisting throughout life on all but the hymenial surface, is a "palisade layer." This layer is formed from club-shaped ends of the hyphae, which arrange themselves closely and evenly at right angles to the surface. In early stages this layer is not well differentiated. Later on it becomes more regular, its threads take on a more pronounced club shape, and the cells become filled with deeply staining contents.

THE MEMBRANE.—The envelope which covers the fruiting body in its early stages arises from the palisade layer. Many of the club-shaped hyphae of the latter continue to grow out beyond the general surface, then turn at right angles, and interlacing in every direction along the surface form a matted web 2–8 threads in thickness. This membrane is very transitory, however, and undergoes degeneration at an early period. Its protoplasm takes on a granular appearance, the cell outlines become indistinct, and finally the whole disintegrates into a deeply staining mass in which the nuclei are the most prominent feature. Long before this process is complete, however, the rapid growth of the underlying tissue bursts the emvelope so that it adheres in flakes (*figs. 57, 58*). Then the paraphyses and intercalary palisade hyphae pushing out to the surface complete the separation and all traces of it are cast off.

DURAND, in speaking of this veil in *Microglossum viride*, states that "when the veil seems to break up into fragments, there are indications that the hymenium first appears through *a pore at the apex*." This phenomenon has been homologized with the opening of the apothecium in Ascobolus. But the coverings of Spathularia, Leotia, and Mitrula break up irregularly without any indication of an apical pore, and in *Helvella elastica* also it breaks irregularly. Moreover, in some forms a veil may be altogether lacking. Indeed I am able to state definitely, from observations on a very complete series of stages, that *Geoglossum hirsutum* shows no trace whatever of such a structure.

THE PARAPHYSES.—In sections of young ascomata of *Helvella elastica* the hymenial surface is not yet differentiated, being quite similar to that in other parts of the fruiting body, and covered by the common palisade layer and external veil. Very early, however, the paraphyses begin to appear, and as these force their way upward

through the palisade filaments the veil is broken and thrown off. The paraphyses arise from ordinary hyphae in the interior of the fruiting body, and are in the early stages quite similar to the filaments of the palisade layer, so that the hymenium can only be distinguished at this period by the greater number and the closer and more regular arrangement of its units. Later on, however, the paraphyses elongate and become thinner, less bulbous, and more closely packed. In this older condition they are filled, especially near their ends, with dense deeply staining contents in which the nuclei seem to have degenerated. They are also septate and their more basal cells show two or more small dense nuclei. The hyphae which give rise to paraphyses may be traced for some distance into the hyphal mass of the cap, and indeed in many cases nearly to its lower surface.

STORAGE BODIES.—A conspicuous feature in sections of *Helvella elastica* is the presence of large cellular bodies filled with contents, and distributed irregularly throughout the whole of the fruiting body except the stem. These bodies are quite large, attaining sometimes 20–30 times the diameter of the surrounding hyphae, but they vary exceedingly in size and shape. Sometimes they form a chain of three or four connected cells, or they may consist of a single mass with irregular protuberances, or they may be merely filaments enlarged for a considerable portion of their length. They often occur in groups, the individuals of which are unconnected.

In most of the ascomata sectioned the protoplasm of these bodies had shrunken somewhat from the cell wall. It is coarsely granular and stains deeply. Nuclei are present in numbers varying from 1 to 20 or more. There are usually present either one or two large nuclei (*figs. 46, 48, 50, 63*), or a number of smaller ones, though both sizes may occur in the same cell (*fig. 46*). Moreover, as shown in *figs. 46–56*, which are taken from a single representative ascoma, all stages intermediate between these extremes may exist. The large nuclei are loosely reticulate in structure, while the smaller are dense and stain deeply. The latter show in many cases a tendency to occur in masses (*figs. 49, 52, 54, 56*). Another conspicuous feature among the nuclei of these large bodies is their frequent arrangement in pairs (*figs. 47, 53, 55, 61*).

The general occurrence of these bodies throughout the cap would

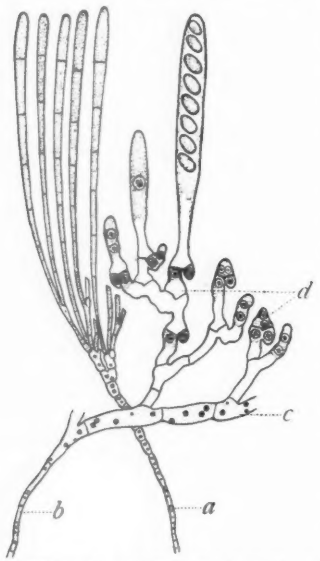
seem to indicate a vegetative function. They are to be found at the base of the hymenium giving off paraphyses, or within the hymenial layer, in which case they are surmounted by one or more paraphyses (*figs. 43, 64*); or they may force their way to the surface, appearing there as enlarged clublike processes plainly having the jointed structure of the paraphyses. The ends of these processes are often found ruptured and the contents, like lava from a crater, overflowing the neighboring surface. These enlargements are further found all through the subhymenial hyphal mass, and many of the palisade hyphae originate from those near the lower surface (*fig. 60*). In addition to that, these bodies are found connected with the fertile filaments either remotely in the subhymenial layer or else having the ascogenous hyphae proceeding directly from them. Often part of an ascogenous hypha, at quite as late a stage as the formation of the hooks, is greatly enlarged, the similarity of stain and nuclear structure leaving no doubt as to the close resemblance of these swellings to the bodies in other parts of the ascoma (*figs. 36, 62*). In very young stages of development these bodies are not present, but they appear quite early in the history of the ascoma, as previously mentioned. At the time when the asci are fully formed, they are usually empty, their connections have vanished, and their walls are shrunken and degenerate. Observations on a considerable number of specimens indicate that these bodies vary with the individual, being largest and most abundant in vigorous plants. They vary also in the individual, serial sections showing one-half of the fruiting body well supplied with them and the other containing scarcely one.

With the foregoing data in hand, it seems most reasonable to regard these peculiar structures as organs for the storage of food material.

ASCOGENOUS HYPHAE.—When the fruiting body is about 1.25^{mm} in diameter, the first hyphae which are undoubtedly ascogenous make their appearance. Some of the hyphal threads form a matted web a short distance below the layer of paraphyses (*fig. 59*). The threads of this web are long, straight, multinucleate, sparsely branched, and twice or thrice the thickness of the ordinary filaments. It is from these that the vertical ascus-bearing threads arise.

A comparison with what DITTRICH records for Mitrula shows that in Helvella there is a much later differentiation of the fertile filaments.

Moreover, they are not traceable to any distinct source, as were those in Mitrula, but arise merely from the ordinary hyphal threads of the fruiting body. This is an agreement with BREFELD'S observations on several species of Morchella, Helvella, and Geoglossum, as well as *Leotia lubrica* and *Peziza Sclerotiorum* (Heft IV, 130). According to him the ascogenous hyphae may arise from the common filaments of the fruiting body and even from those which had formerly produced paraphyses.



Hymenium of *Helvella elastica* (semi-diagrammatic): *a*, sterile hypha producing paraphyses; *b*, undifferentiated fertile filament; *c*, enlarged fertile filament in the sub-hymenial layer; *d*, ascogenous hyphae showing the succession of hooks, the fusion of terminal and antepenultimate cells, and the formation of the ascus (the unshaded parts are empty).

In *Helvella elastica* there is frequently a thickening of the sterile filaments before they form the profusely branched mass of paraphyses, but such enlargements have no connection with those occurring in the ascogenous hyphae. The cells of the former are short, bulbous, and usually binucleate, while in the fertile threads they are long, uniform, and multinucleate (see *text fig.*). Moreover, no connection can be traced between the two, nor can any paraphyses be seen coming from the ascogenous filaments.

From the fertile subhymenial layer lateral branches are given off in rather scanty numbers, and these grow toward the layer of paraphyses, where they branch repeatedly. The end cells of these lateral branches contain an abundance of deeply staining protoplasm and 2–5 nuclei, the usual number, however, being 2 (*figs. 11–19*). The whole energy of growth seems to be concentrated in this end cell, for usually those behind it are completely empty, and long before branching has been completed even the walls disappear. DITTRICH notes

the same phenomenon in *Mitrula phalloides*. It is evident that in these cases the developing ascus must be nourished by diffusion from the surrounding cells. As growth proceeds in the vertical branch, transverse walls are formed at intervals along its course (*figs. 11–18*). When nuclei and protoplasm happen to be cut off by these walls, a lateral branch may be produced from the cells thus left behind (*figs. 12, 16*). Finally the end cell forms a hook and its two nuclei divide to form four. Then follows a septation of the hook into three cells, a daughter of each of the mother nuclei remaining in the penultimate cell and the other two being distributed to the terminal and antepenultimate respectively. From the penultimate cell a process now arises into which its two nuclei wander (*figs. 20, 27.*)

Heretofore in the Ascomycetes which have been investigated there has come into consideration only one hook (where hooks exist), the process from which becomes the ascus. Here, however, it is curious to note that the process from the first hook may proceed to form a second. Its two nuclei, instead of fusing, divide again, the resulting four being distributed in the second hook as in the first. Indeed this formation of successive hooks may go on for some time, as many as six having been observed on one ascogenous hypha. The last-formed process then becomes the ascus, its nuclei fusing to form the primary ascus nucleus, and from this spore nuclei are produced by the usual three successive divisions. One important feature is made strikingly evident by these hooks, namely the conjugate division of the nuclei in the ascogenous hyphae. It has been quite evident from my observations that the two nuclei which fuse in the young ascus are directly descended by conjugate division from the two which normally occupy the end cell of the ascogenous hypha as it first arises from the subhymenial layer.

Another phenomenon of interest is the "fusion" or "anastomosis" that quite frequently takes place between the terminal and antepenultimate cells. The nucleus of the antepenultimate cell passes into the former, and from thence the two nuclei wander into a process which invariably arises from the terminal cell (*figs. 33, 34, 36–38, 40*). It will be readily seen that this process with its two nuclei is the direct equivalent of that which originates from the penultimate cell, and indeed it conducts itself in exactly the same way, forming

further series of hooks in which anastomoses may likewise take place, all the resulting processes finally ending in asci (*text fig.* and *figs. 32, 38, 40–42*).

Occasionally there has been obviously no fusion of the terminal and antepenultimate cells, yet a branch has arisen from the former. As in such cases the antepenultimate cell retains no nucleus, it is probable that pairing of the daughter nuclei has taken place before the cutting off of a binucleate penultimate cell, a case quite comparable to that observed by MAIRE (**15**) in *Galactinia succosa*.

Summary

The mycelium of *Helvella elastica* is subterranean. Its cells are multinucleate.

The fruiting body arises from clumps of thick short-celled filaments. This fruiting body is quite early surrounded by an envelope which is transitory and breaks up irregularly. Closely associated with the veil in early stages, but persisting throughout the life of the fruiting body, is an external layer of club-shaped palisade hyphae.

Certain large irregular cells occurring in all parts of the fruiting body (except the stem) apparently have the function of storage organs.

The "ascogenous hyphae" arise as a clearly differentiated subhymenial complex of filaments. From this layer grow out vertical branches whose end cells contain usually two nuclei. Hooks are formed by these end cells, while the proximal part of the branch degenerates.

The process from the first hook, without fusion of its two nuclei, may proceed to form a second and similar hook. From this second a third may arise, and so on, up to the number of at least six, the process from the last becoming the ascus after nuclear fusion.

In any hook of such a series a "fusion" or "anastomosis" of the terminal and antepenultimate cells may take place, the nuclei of both passing into a process arising from the former. This process is equivalent to that springing from the penultimate cell, and conducts itself in the same way.

The two nuclei which fuse to form the primary ascus nucleus are directly descended by conjugate division from the two which occupied the end cell of the young ascogenous hypha.

No structure having the conventional form of an ascogonium was found.

UNIVERSITY OF TORONTO

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EXPLANATION OF PLATES XIV–XVI

FIGS. 1–5.—Parts of mycelium. ×300.

FIGS. 6–10.—Stages showing development; actual length indicated.

FIGS. 11–19.—Young ascogenous hyphae before formation of hooks. ×360.

FIGS. 20–27.—Formation of first hooks. ×360.

FIG. 28.—Young ascogenous hyphae arising from enlarged filament in the subhymenial layer. ×360.

FIGS. 29–31.—Formation of successive hooks. ×360.

FIGS. 32–34.—Fusion of antepenultimate and terminal cells. ×360.

FIG. 35.—Drawing from maceration showing three successive hooks ending in young ascus.

FIG. 36.—Enlarged ascogenous hypha, the enlargement involving four successive hooks. ×360.

FIG. 37.—Swollen hook from same group as *fig. 36*, showing fusion of terminal and antepenultimate cells.

FIGS. 38, 40–42.—Fusion of terminal and antepenultimate cells.

FIG. 43.—Storage body in paraphyses layer.

FIGS. 44, 45.—Storage bodies from palisade layer on under surface of cap. ×360.

FIGS. 46–56.—Storage bodies in interior of the ascoma showing size and arrangement of the nuclei. ×360.

FIG. 57.—Young fruiting body with remnants of the envelope adhering. ×60.

FIG. 78.—Part of surface of *fig. 57*, showing palisade layer and fragments of the membrane. ×300.

FIG. 59.—Section through the cap. ×100.

FIG. 60.—Filaments of the palisade layer arising from storage bodies. ×300.

FIG. 61.—Storage bodies (outlined in ink) showing paired nuclei. ×300.

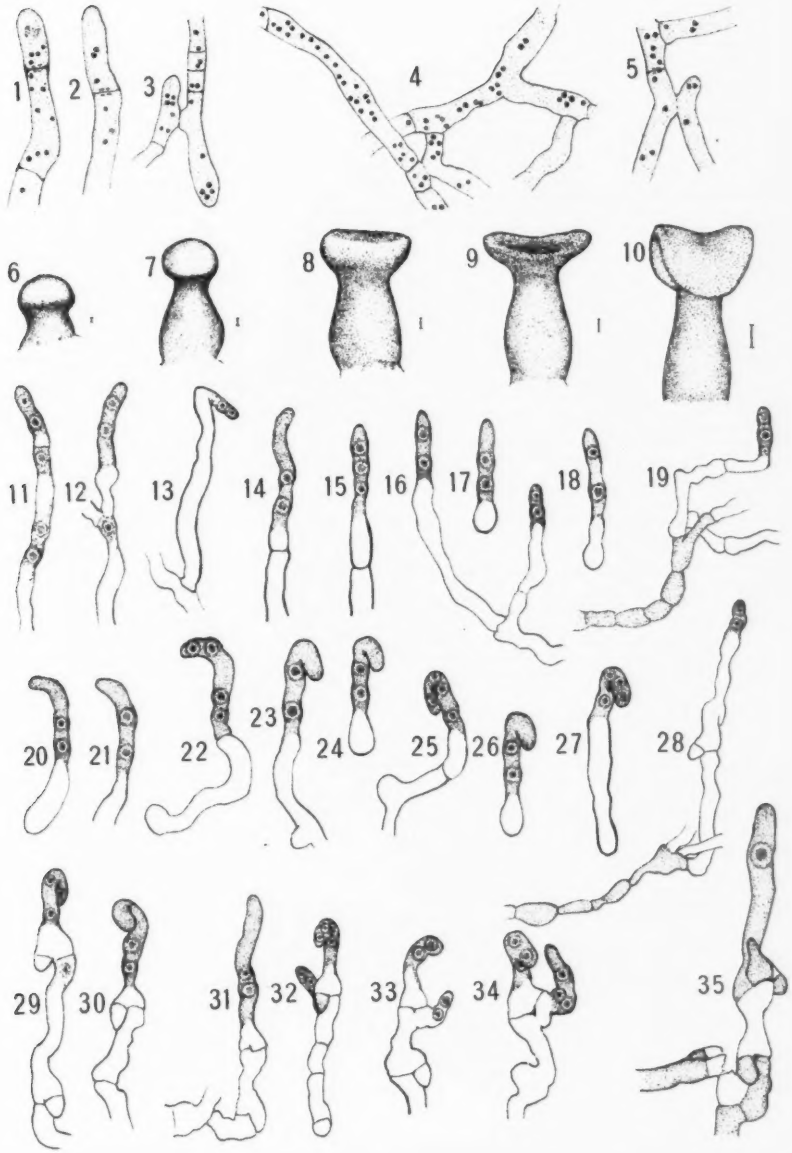
FIG. 62.—Enlargement of ascogenous hypha involving several hooks (see *fig. 36*).

FIG. 63.—Storage cell with two large and several small nuclei (*fig. 46*).

FIG. 64.—Storage bodies in the hymenial layer ending in paraphyses.

FIG. 65.—Young ascogenous hypha with two successive hooks.

FIG. 66.—Section of young fruiting body with concave hymenial surface.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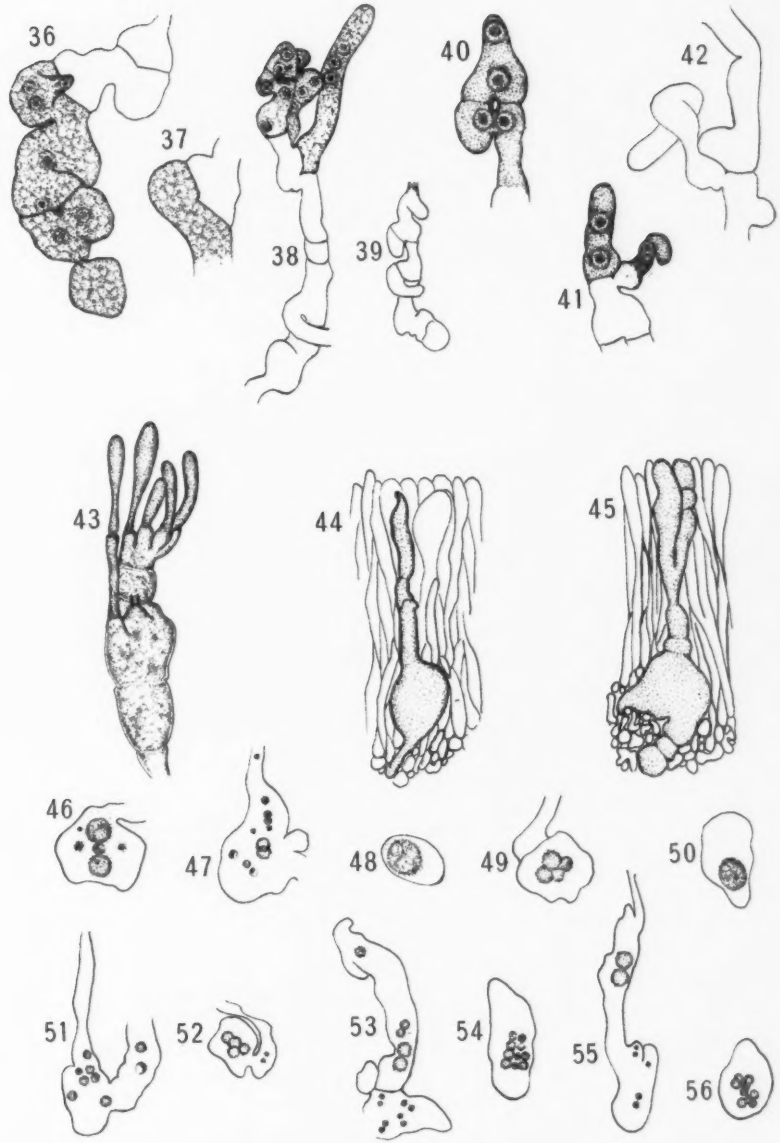
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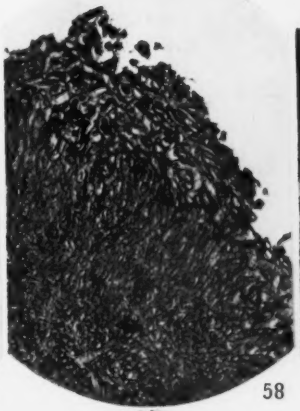
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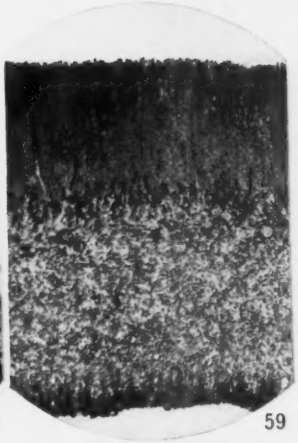
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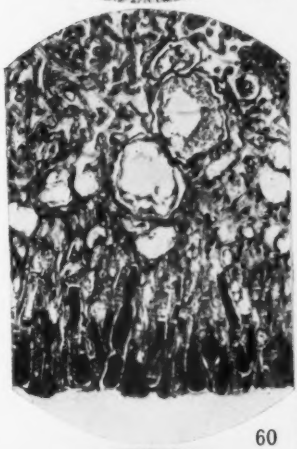
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McCUBBIN on HELVELLINEAE

ON PHYSIOLOGICALLY BALANCED SOLUTIONS FOR BACTERIA (B. SUBTILIS)

CHARLES B. LIPMAN

In a former paper[1] I have shown that the laws governing the toxic and anti-toxic effects of salts, which LOEB and OSTERHOUT have found to hold so universally for animals and plants respectively, hold also for bacteria if we may consider *B. subtilis* as representative of this group. I shall now attempt to show that as regards complete physiologically balanced solutions, the same general relation holds for bacteria as for animals and plants.

In his experiments on a marine fish (Fundulus) LOEB was able to show that the views of HERBST regarding the absolute necessity for each of the constituents of sea water for the complete development of the egg were erroneous, since Fundulus, which would not develop in a pure NaCl solution of the same osmotic pressure as sea water, would live indefinitely in distilled water, and therefore the salts contained in sea water were necessary, not for nutrient purposes, but for the purpose of overcoming toxic effects of other salts, or briefly for balancing purposes. From this and other experiments in which he was able to overcome the toxicity of NaCl solutions by the addition of small amounts of salts like $ZnSO_4$, $BaCl_2$, and Pb $(CH_3COO)_2$, which are themselves exceedingly toxic, LOEB concluded that physiologically balanced solutions, in which one constituent overcomes the toxic effect of one or more others, are essential for the proper development of animals, and further that the blood and sea water may be considered such balanced solutions in nature.

In similar experiments on plants OSTERHOUT[2] showed that LOEB'S conception of "physiologically balanced solutions" holds good also for that class of organisms. In many series of tests on a great variety of marine and freshwater plants that investigator proved that the combination of NaCl, KCl, and $CaCl_2$ in the proportions in which they exist in sea water is just as uniformly beneficial for plants as for animals.

[1] BOT. GAZETTE **48**:105–125. 1909.

[2] Univ. Calif. Publ. Bot. **2**:no. 10. 1906.

From the interesting results just cited, it was deemed important to ascertain if, contrary to the opinion of LOEW and ASO,[3] physiologically balanced solutions are necessary for bacteria as they have been found to be for plants and animals, and if the behavior of bacteria toward completely balanced solutions is in full accord with that of the other classes of organisms. Accordingly, seven series of experiments were carried out, in which were employed upward of thirty solutions of different composition. In order to have the results strictly comparable, the average of several determinations in each case is inserted in the tables. It may be said that the duplicates showed very close agreement for ammonification cultures. The technique employed in these experiments was in general the same as that described in my former experiments above cited. The cultures were incubated in Erlenmeyer flasks of 250^{cc} capacity for 2.5 days, at the end of which time the ammonia was determined. The solutions in each flask consisted of the constituents described under each in the tables, and, in order to provide uniform surfaces of aeration, were reduced to a bulk of 100^{cc} in each flask after the salts were combined in the proportions given.

SERIES I

The object here was to compare pure solutions of NaCl and KCl and combinations of them with $MgCl_2$ and $CaCl_2$ respectively (which combinations have been shown to be beneficial in the experiments with binary solutions) with a blank solution of peptone in distilled water and one of peptone in tap water. The results are given in table I.

In this series, as well as in former experiments, the salt mixtures which contained NaCl as the predominant salt always seemed to give better results, and it was decided therefore in the following series to make extensive use of the NaCl as a basis for all artificial solutions. We see from the table that the best results obtained with the salt solutions was in solution 3, as was the case in the experiments with binary solutions, but it will also be noticed that the blank peptone solutions in both tap and distilled water gave very much higher results than even solution 3. The depressing effect seen in the tap

[3] Bull. Coll. Agric. Imp. Univ. Tokyo **7**:355. 1907.

water peptone solution when compared with the distilled water culture is possibly due to the fact that small quantities of toxic salts

TABLE I

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.91 PER CENT.

Number	Culture solution	N as NH_3 formed in cultures, in mg
1	100 NaCl	20.10
2	100 KCl	15.38
3	100 NaCl + 10 $MgCl_2$	23.39
4	100 KCl + 5 $CaCl_2$	17.90
5	Peptone in distilled water	42.51
6	Peptone in tap water	31.90

are dissolved from the metal pipes by the water and thus have an unfavorable effect on the growth and development of *B. subtilis*.

SERIES II

The next step taken was to ascertain if salt mixtures containing three salts instead of two provide better conditions for ammonification

TABLE II

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.91 PER CENT.

Number	Culture solutions	N as NH_3 formed in cultures, in mg
1	100 NaCl + 10 $MgCl_2$ + 10 KCl	20.63
2	100 NaCl + 10 $MgCl_2$ + 10 $CaCl_2$	18.91
3	100 NaCl + 10 $MgCl_2$ + 5 $CaCl_2$	25.88
4	100 NaCl + 10 $MgCl_2$ + 25 KCl	24.90
5	100 NaCl + 10 $MgCl_2$ + 50 KCl	22.10
6	100 NaCl + 10 $MgCl_2$	23.39

by *B. subtilis* in peptone solutions. The solutions were prepared as shown in table II, and the ammonia determined with results as given.

Comparing the first five solutions with solution 6, we find that we have not yet arrived at what might be termed a balanced solution. Some salt mixtures of three salts do not give as good results as solution 6, while solutions 3 and 4, which appear to be better than the former, are not sufficiently near the peptone water cultures (see table I) to allow them to stand without further experiments as the optimum solutions. None the less, it is plain that solution 3 is approaching the balanced solution.

SERIES III

From the results just given it was thought that the addition of another salt to the mixtures of three salts might tend to make them

TABLE III

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.01 PER CENT.

Number	Culture solution	N as NH_3 formed in cultures, in mg
1	100 NaCl, 10 $MgCl_2$, 10 $CaCl_2$, 10 KCl	19.37
2	100 NaCl, 10 $MgCl_2$, 5 $CaCl_2$, 10 KCl	18.91
3	100 NaCl, 5 $CaCl_2$, 10 $MgCl_2$, 25 KCl	21.75
4	100 NaCl, 10 $CaCl_2$, 10 $MgCl_2$, 25 KCl	22.87
5	100 NaCl, 10 $MgCl_2$, 5 $CaCl_2$	25.88
6	100 NaCl, 10 $MgCl_2$	23.39

approach still closer than did solution 3 of the last series the optimum solution. There were prepared, therefore, solutions containing

various combinations of four salts each, and for comparison with them solution 3 of table II containing three salts and solution 6 of table II containing only two salts.

From table III it is plain that the various combinations of four salts, though allowing a fairly good production of ammonia, are not as favorable to *B. subtilis* as either 5 or 6, which contain respectively only three and two salts. It appears evident that the combinations of four salts there employed have not the favorable composition of a balanced solution, and since it could not easily be ascertained as to what was the depressing agent in these solutions, it was decided to run another series with different combinations of four salts each, to see if any other combination of four salts can more nearly be made to approach the balanced solution.

SERIES IV

Since calcium proved to be so toxic a salt to *B. subtilis*,[4] it was thought that an improvement could be made in the combinations of four salts by using smaller amounts of calcium than were employed in the last series, and solutions of four salts each (shown in table IV)

TABLE IV

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.01 PER CENT.

Number	Culture solutions	N as NH_3 formed in cultures, in mg
1	100 NaCl, 25 KCl, 10 $MgCl_2$, 2 $CaCl_2$	20.70
2	100 NaCl, 25 KCl, 10 $MgCl_2$, 1 $CaCl_2$	21.12
3	100 NaCl, 10 KCl, 10 $MgCl_2$, 2 $CaCl_2$	28.42
4	100 NaCl, 10 $MgCl_2$, 5 $CaCl_2$	25.88
5	Peptone in distilled water	42.51
6	Peptone in tap water	31.90

[4] LIPMAN, CHAS. B., BOT. GAZETTE **48**:105–125. 1909.

were arranged and compared with solution 5 of the last series containing three salts and with a solution of peptone in distilled water and one in tap water.

The large proportion of the potassium salt interfered with the beneficial action of the calcium in solutions 1 and 2, as can be seen by comparing them with solution 3, which differed from the others only in having less potassium and in consequence seems to approach quite closely the peptone solution in tap water, and also is far better than solution 4, which until now showed up as the most favorable solution of all the artificial salt mixtures employed. Solution 5 as before shows the optimum conditions, since there is neither high osmotic pressure nor any toxicity in it to interfere with the ammonification by *B. subtilis*.

SERIES V

Since in the foregoing series we have been gradually approaching a balanced solution in some of the salt mixtures, it is appropriate to compare them now with sea water, both in its original and diluted forms, as well as with RINGER'S solution (which consists of 100 parts NaCl, plus two parts KCl, plus two parts $CaCl_2$), and with a solution

TABLE V

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS, EXCEPT AS EXPLAINED ABOVE

Number	Culture solution	N as NH_3 formed in cultures, in mg
1	100 NaCl 10 $MgCl_2$ 5 $CaCl_2$	25.88
2	100 NaCl 10 KCl 10 $MgCl_2$ 2 $CaCl_2$	28.42
3	RINGER'S solution	19.74
4	Natural sea water	10.72
5	Dilute sea water	31.35
6	Artificial sea water (VANT' HOFF'S solution)	31.41

made up so as to constitute an artificial sea water (VAN'T HOFF'S solution). The natural sea water was obtained from Pacific Grove, California, and by titration for chlorids was found to have a con-

centration of 0.53 *m with an alkaline reaction.* The dilute sea water was made by adding distilled water to the concentrated sea water until a concentration of 0.35 *m* (or that used in all the other solutions) was obtained. The artificial sea water is VAN'T HOFF'S solution and is neutral in reaction, thus according with the reaction of all other solutions employed.

The superiority of the sea water solutions whose concentration does not exceed 0.35 *m* can be easily seen from table V. It is plain that the artificial sea water and the natural sea water of the same concentration are by far the most superior of any salt mixtures tried, and though in the last table it is compared with the most favorable salt mixtures and those which appear to approach most closely to a balanced solution, it excels even those to quite a marked extent. RINGER'S solution, not having the magnesium salt which seems to act so beneficially in balancing solutions for *B. subtilis*, gives only a fair ammonification coefficient as compared with other salt mixtures.

SERIES VI

It seemed of interest to see what effect the addition of magnesium to the RINGER'S solution would have, and a series was therefore arranged to show this.

TABLE VI

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.01 PER CENT.

Number	Culture solution	N as NH_3 formed in cultures, in mg
1	RINGER'S solution	19.74
2	RINGER'S solution + 10 $MgCl_2$	26.44
3	Artificial sea water	31.41
4	Peptone in brook water	39.80
5	Peptone in distilled water	42.51
6	100 NaCl, 10 $MgCl_2$, 10 KCl, 2 $CaCl_2$	28.42

It is quite evident that the addition of magnesium to RINGER'S solution has a very favorable effect, and by slightly changing the proportion of the constituents we could easily obtain what appears

here to be the optimal solution. The brook water solution was inserted here to add another solution to the number of those which allow so vigorous an ammonification owing to the low osmotic pressure. The artificial sea water, however, still remains far superior to any other salt mixture thus far tried.

SERIES VII

In order to compare to the best advantage the various salt mixtures with the artificial sea water and distilled water solutions, it was decided to arrange in one series the best representatives of the single, binary, ternary, and quaternary salt solutions, along with the artificial sea water and the distilled water peptone solutions.

TABLE VII

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS
PEPTONE CONTENT 0.01 PER CENT.

Number	Culture solution	N as NH_3 formed in cultures, in mg
1	100 NaCl	20.10
2	100 NaCl 10 $MgCl_2$	23.39
3	100 NaCl 10 $MgCl_2$ 5 $CaCl_2$	25.88
4	100 NaCl 10 $MgCl_2$ 10 KCl 2 $CaCl_2$	28.42
5	Artificial sea water	31.41
6	Peptone in distilled water	42.51

We see here to good advantage the beneficial effects of balancing solutions. Of the large variety of single salts and combinations of salts of various kinds, there was none that could quite approach in efficiency the artificial sea water or the natural sea water of the same concentration, and we are obliged to accept the fact that for bacteria (or at least for that group represented by *B. subtilis*) physiologically balanced solutions are necessary if optimal development of the bacteria is sought for.

DISCUSSION OF RESULTS

The number of combinations of salts that could be experimented with in a manner similar to that above given is of course unlimited,

and it is impossible to make determinations of all of them, but in the foregoing series of experiments enough combinations have been tried and sufficient evidence has been adduced to make it quite clear that, for at least one class of bacteria, sea water is a physiologically balanced solution just as truly as it is for animals and the higher plants. Therefore, although some of the results exhibit some puzzling aspects, the prime object of these investigations—to discover whether sea water or other balanced solutions are superior to unbalanced solutions—is attained. The inexplicable facts which are bound to arise in such investigations must be accounted for by future research, but the universality of LOEB'S conception of "physiologically balanced solutions" is more firmly grounded than ever. To the experimental proof of it for animals in the ingenious experiments of LOEB himself are added the no less remarkable results of OSTERHOUT[5] in his work on marine as well as freshwater and terrestrial plants, and to both of these are now added the results above described on the third great class of living organisms which seem to hold the same general relation to balanced solutions as the other two.

SUMMARY

1. The ammonifying power of *B. subtilis* is stronger in artificial sea water or in natural sea water of the same concentration than in any other salt mixture.

2. Sea water may be looked upon therefore as a physiologically balanced solution for *B. subtilis* as truly as it is for animals and the higher plants.

I am indebted to Professor W. J. V. OSTERHOUT of Harvard University for helpful suggestions and critical reading of the manuscript.

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[5] BOT. GAZETTE **42**:127–134. 1906; **44**:259–272. 1907.

BRIEFER ARTICLES

THE PHYSIOLOGICAL CONDITIONS FOR THE DEVELOPMENT OF MONOECIOUS PROTHALLIA IN ONOCLEA STRUTHIOPTERIS[1]

The following investigation was undertaken with the view of ascertaining the validity of the statement made by CAMPBELL concerning the dioeciousness of *Onoclea Struthiopteris*. He says: "In the genus Onoclea, as well as some other Polypodiaceae, the prothallia are regularly dioecious, and only a part of them develop the archegonial meristem. The others remain one-layered, and are often of very irregular form, and may be reduced to a short row of a few cells. All of the 'ameristic prothallia,' as PRANTL calls them, are males. In the majority of the Polypodiaceae these occur more or less plentifully, and are often the result of insufficient nutrition; but in Onoclea it is something more than this, as not only the small prothallia are males, but the large ones are exclusively females, and not hermaphrodite, as in most ferns."[2]

The development of fern prothallia, and their reproductive organs, has been studied until recently more from the morphological than the physiological point of view. Much of the work of physiological importance demonstrates the effect of various light intensities. PRANTL (in 1881) was one of the first to investigate carefully the effect of nutrition. His observations upon cultures of *Polypodium vulgare*, *Aspidium filix-mas*, *Ceratopteris thalictroides*, and *Osmunda regalis* led him to draw the following conclusions: that the food supply determines the development of the prothallia and the separation of the sexual organs; that good cultural conditions favor the production of meristem and the development of archegonia; that poor cultural conditions retard the production of meristem and favor the development of antheridia; that as nitrogen is one of the requisites of a good cultural condition, an insufficient nitrogen supply may be the cause of thickly grouped prothallia bearing only antheridia.

The spores of *Onoclea Struthiopteris* used in the experimental work were collected from yards about Ann Arbor in September and sown soon after

[1] Contribution No. 114 from the Botanical Department of the University of Michigan.

[2] Mosses and ferns. Edition 2. p. 314.

collected. Cultures were made upon soil in porous clay pots, and in sterilized distilled water.

Germination began in the soil cultures, four to six days after the spores were sown. Six weeks later, antheridia and archegonia appeared. As the genus Onoclea has been considered by most writers to be dioecious, it was interesting to find monoecious prothallia occurring in about 1 per cent. of the soil cultures; the other prothallia seemed regularly dioecious. The monoecious prothallia were of medium size, slightly heart-shaped, with a meristem bearing the archegonia; the antheridia, fewer in number than the archegonia, developed from the marginal cells. Some of the dioecious prothallia were large, heart-shaped, with a meristem bearing many archegonia; others were smaller, filamentous, slightly heart-shaped, or very irregularly shaped. These prothallia were one layer of cells in thickness, lacked a meristem, and the antheridia were formed both from the marginal and ventral cells.

The development of the prothallia did not seem entirely influenced by their position, as indicated by PRANTL, for many of the smaller prothallia, the "ameristic" ones, were found in the less crowded regions; while the larger prothallia, the "meristic" ones, bearing only archegonia, were often found in the more crowded regions.

An attempt was made to produce monoecious prothallia through the influence of an inorganic solution. For this purpose large, heart-shaped prothallia with archegonia in various stages of development were selected and transferred from the soil to BEYERINCK'S solution. Four to seven days later, in one-half of the cultures examined, a number of monoecious prothallia were found, antheridia having developed from the marginal cells of the prothallia, near the notch. In all cases, death of the prothallia immediately followed, which seemed to be due in some cases to too long exposure to direct sunlight, in others to injury of tissue in transferring from the soil to the solution.

Other cultures were transferred from the sterilized distilled water to solutions and kept in moderately weakened light. Two to four weeks later, in these cultures, *the majority of the prothallia became monoecious;* numerous antheridia were produced, both from marginal cells and from the ventral surface cells on the sides, and near the apex of the prothallia. Other prothallia gave rise to numerous proliferations; some were mere filaments bearing antheridia at their apices; others had the thallus form, were distinctly notched, and produced antheridia from the marginal and ventral surface cells.

After all the prothallia needed for experimental purposes had been

removed, the pots were set aside and neglected for about two weeks. At the end of this time, the surface of the soil was brown, and all the prothallia appeared dead. The pots were then kept moist under bell jars in weakened light. After a period varying from six to eight weeks, many of the old prothallia had developed adventitious outgrowths. Some of these outgrowths were filaments or irregularly shaped prothallia covered with antheridia. Others developed into elongated prothallia bearing archegonia, which in turn frequently became monoecious by developing adventitious shoots bearing antheridia.

Germination began in the sterilized water cultures four days after the spores were sown. Six days later the prothallia had attained a growth of ten cells. A set of cultures was then made, by transferring 100 prothallia to each of several nutritive solutions. For each of these sets a control set was kept in sterilized distilled water. Six weeks after the prothallia had been transferred to the nutritive solutions, male prothallia bearing antheridia and female prothallia bearing archegonia were observed in about equal proportions. The prothallia were regularly dioecious, and at this time no monoecious prothallia were observed. That these antheridia and archegonia were functional is shown by the production of vigorous young sporophytes. One of these sporophytes lived five months in KNOP'S solution, and developed five fronds, one of which was 2.5^{cm} in length. Twelve weeks after the transference of the prothallia to the nutritive solutions, many monoecious ones were observed in the cultures of KNOP'S solution. These first developed the meristem with archegonia, afterward antheridia were formed from the marginal and ventral cells of the adventitious outgrowths.

CAMPBELL (*l. c.* 321) makes the statement that "as soon as an archegonium is fertilized, no new ones form, but it frequently happens that a very large number prove abortive before finally fertilization is effected." In all the cultures examined, both from the soil and the nutritive solutions, frequently prothallia have been observed producing new archegonia after fertilization had taken place. In the solution cultures young archegonia, antheridia, and sporophytes were present at the same time upon the same prothallia.

A further attempt to produce monoeciousness was made, by transferring prothallia bearing archegonia from one nutritive solution to another. Results were obtained in the following cultures: (*a*) from KNOP'S solution to BEYERINCK'S solution, lacking potassium dihydrogen phosphate; (*b*) from SACHS'S solution with 1 per cent. potassium phosphate, to KNOP'S solution. In these cultures, three to four weeks after the transfer had

been made, monoecious prothallia were observed. The prothallia continued to grow for ten to eleven weeks and developed many adventitious outgrowths bearing antheridia. Young sporophytes were also developed.

In summing up, therefore, it may be said: monoecious prothallia, as well as dioecious prothallia, were observed in soil cultures; monoecious prothallia were obtained from "female" prothallia transferred at a ten-celled stage from distilled water to KNOP's solution; monoecious prothallia were obtained by transferring "female" prothallia from the soil to a nutritive solution; they were also obtained by transferring prothallia from one nutritive solution to another.

The experimental work of this paper was done in the botanical laboratory of the University of Michigan. I wish to express my indebtedness to Dr. C. H. KAUFFMAN, who suggested the problem and under whose guidance and inspiration the work was carried on.—ELIZABETH DOROTHY WUIST, *University of Michigan.*

CECIDOLOGY IN AMERICA

Botanists are usually interested in the malformation of plants, and some of the earliest literature contains references to these abnormal structures. However, cecidology is one of the latest of the biological sciences to be developed. The literature is so involved with other branches of biology that only those who have given special attention to the subject can have a very clear conception of the dimensions of modern cecidology and the character of its problems. The entomologists have given far more attention to the subject, in both Europe and America, than any other group of scientists. For this reason many have come to consider cecidology as a branch of entomology, while as a matter of fact the entomologist has much greater interest in the insects than in the galls.

Cecidology now includes all hypertrophies caused by specific organisms, as insects, nematodes, fungi, and bacteria. It has been suggested that the term "gall," which has been in use since the sixteenth century, be discontinued and the word *cecidium* be substituted; also that the structures caused by these different organisms be designated as diptero-cecidia, phyto-cecidia, nemato-cecidia, myco-cecidia, etc. Some writers have been inclined to include that very much confused and mysterious class of diseases known as teratology under the head of cecidology, and there appears to be no conclusive argument as to why it should or should not be so considered.

The early literature is so thoroughly involved with other subjects as to be practically unavailable to the general students, many valuable references

being found in papers upon other subjects, with nothing whatever in the titles to indicate their presence. For this reason many workers in both botany and entomology have frequently thrown aside interesting material.

A brief résumé of the subject may be of interest to the students of botany. Very little work was done and very few papers published before 1861, when C. R. VON OSTEN-SACKEN, of the foreign diplomatic service in Washington, began the publication of a series of papers which appeared in the Proceedings of the Entomological Society of Philadelphia, various U. S. government publications, Smithsonian Institution publications, etc. Since that time many entomologists have published valuable papers on this subject and also many important notes in connection with papers on other phases of entomology. Up to the present time there have been about 125 workers who have given some attention to this subject in North America, and they have published approximately 350 papers and notes.

In Europe the subject is very little older than in America, but has attracted the attention of far more workers. Although there have been approximately 350 workers, who have issued about 1200 publications, not more than 100 were published before 1861, which date may be considered the beginning of the subject in this country. It is also true that the greater part of the work in Europe has been done by the entomologists, but in recent years a great deal of attention has been devoted to the study of the cecidia themselves.

From the viewpoint of the botanist, cecidology presents a most interesting field for research. Both botanist and entomologist are continually confusing the cecidia caused by fungi and insects, and the confused state of the literature has led to the neglect or abandonment of many interesting problems. The fact that most of the work has been done by the entomologist has made the literature somewhat inaccessible to the botanist, and has also given the younger botanists a misconception of the character of the subject.

Not only has the confusion caused the neglect and abandonment of problems, but has caused some rather interesting and amusing statements as the result of misinterpretations. This may be illustrated by *Bucida buceras*, a tropical plant which produces two kinds of fruits, one small and the other long. Both names (*Bucida* proposed by LINNAEUS in 1759 and *buceras* by P. BROWNE in 1756) are derived from the long fruits, which authors considered as resembling the horn of a bull. However, recent studies have shown that the long fruits were hypertrophied as the result of mite injuries.[3]

Cecidology is not only closely interwoven with entomology, but also

[3] BRITTON, N. L., The generic name Bucida; and COOK, MEL. T., The hypertrophied fruit of *Bucida buceras*. Bull. Torr. Bot. Club **35**:303–306. 1908.

with mycology, bacteriology, plant pathology, and plant physiology. The connection with entomology is fully appreciated and needs no comment at this time. Many fungi produce cecidia, but the character of these cecidia have received as little or less attention from the mycologist than the insect cecidia from the entomologist. The cecidia on the roots of legumes and the recent investigations of SMITH and TOWNSEND on crown galls demonstrate the relationship to bacteriology. Since the cecidia from whatever cause are pathological conditions of the plants, cecidology becomes a well-defined part of plant pathology. Until recently, plant pathology has been more especially interested with the cause and control of diseases, in fact has been a branch of mycology. However, with the further progress of the subject we must come more and more to recognize the necessity of a study of the pathological condition of the plant. This feature of the work will involve the most careful and exact methods of the plant physiologist. However, the connection of cecidology with physiology is not only through pathology, but direct. There is that long-mooted question concerning the character of the stimuli in the formation of cecidia, which has never been answered satisfactorily.

An explanation of the character of the stimuli in the formation of myco-cecidia might give some aid in answering the question, and this latter problem is undoubtedly within the province of plant physiology. The explanation of why injuries result in hypertrophies in some cases and atrophies in others is also an interesting problem for some ambitious student of botany. The fact that "the morphological character of the gall depends upon the genus of the insect producing it rather than upon the plant on which it is produced," as demonstrated by the writer,[4] suggests an interesting field of investigation. The so-called evolution of the cecidia may be a response to physiological conditions rather than to evolutionary factors.

The distribution of cecidia as compared with the distribution of the host plant furnishes a good supply of work for the ecologist, while the injurious character and valuable properties of many cecidia will give profitable fields for the economic phases of the biological sciences.

The greatest need of cecidology in America at the present time is the cataloguing and indexing of the literature and the indexing of the cecidia with reference both to the causes and to the host plants. To this should be added up-to-date, available descriptions of the cecidia and the organisms which cause them. Some work along this line has been done by Mr. WM. BEUTENMÜLLER of the American Museum of Natural History, and by the writer.

[4] COOK, MEL. T., Galls and insects producing them. Ohio Naturalist 2:263–278. 1901.

There is at the present time one international publication devoted entirely to cecidology, *Marcellia*, now in its eighth volume, edited by Dr. A. TROTTER, Avellino, Italy, which publishes original papers and also current bibliography.— MEL. T. COOK, *Delaware Agric. Exper. Station, Newark.*

FIXING AND STAINING TANNIN IN PLANT TISSUES WITH NITROUS ETHERS

(WITH EIGHT FIGURES)

Immature dates exposed to the vapor of amyl or ethyl nitrite to stimulate premature ripening were observed to turn dark brown very rapidly. This was found to result from the staining of the giant tannin cells which occur near the cuticle. After several hours' exposure, hard tannin grains were formed in the tannin cells of green dates and persimmons, which had the same physical characters as the grains occurring naturally in ripe fruit; they could be separated quite pure by gravity in water, and when pressed beneath the cover glass fractured like grains of gelatin.

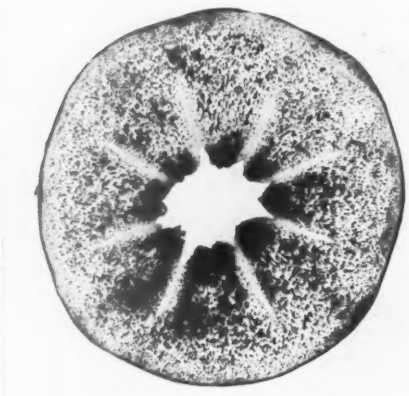
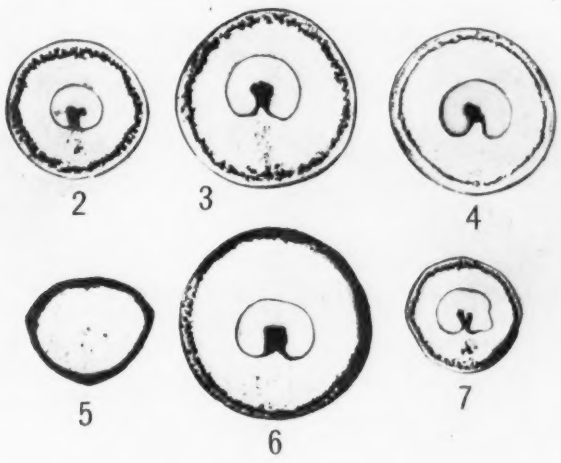


FIG. 1.—Japanese persimmon.

The juice of the unripe date gives a dense brown precipitate with ethyl or amyl nitrite or with sodium nitrite and free acid. Persimmon juice gives an intense deep wine-red color, but no precipitate. Tannic and gallic acids give yellow, while phloroglucin and some other higher phenols give a red color very similar to that given by date or persimmon juice. Phloroglucin also gives a precipitate, but it does not correspond in appearance with that given by date juice. The other higher phenols also give yellow or red color reactions with nitrous ether. The gummy matter, pectin, etc., precipitated from the juice by alcohol carries with it much of the substance that reacts with the nitrite, and the color given by this pre-

cipitate increases by repeated precipitations. Date juice obtained under low pressure continues for many weeks to give the ethyl nitrite reaction, as also the reactions with formaldehyde and ferric chlorid, but juices obtained under very high pressure, after standing, lose the property of giving these reactions, excepting that with ethyl nitrite a very much lighter colored precipitate is formed. DAVY[5] found the color reaction between gallic acid and nitrous acid very delicate, and applied it to the colorimetric estimation of nitrites.



FIGS. 2–7.—*Fig. 2*, Deglet Noor date; *fig. 3*, Oga de Bedreschen; *fig. 4*, Birket el Haggi; *fig. 5*, seedling unfertilized; *fig. 6*, same seedling fertilized; *fig. 7*, M'Kentichie Degla.

It is thus not easily proven whether the precipitate formed in the date juice is due to date tannin alone or whether other substances enter the reaction. Tannin or its derivatives, however, are essential to the reaction, as shown by the following evidence. Only those cells in the date are stained by ethyl nitrite which have been shown to carry tannin by the ferric chlorid, ammonium molybdate, and cupric acetate reactions. Ripe date juice no longer gives the reaction, and immature fruits lose their astringency after very moderate treatment with ethyl nitrite. Juices treated with lead subacetate, gelatin acid salt solution, and hide powder no longer

[5] Through J. DEKKER, De Looistoffen II. p. 44. Amsterdam. 1908.

give the reaction. The lead subacetate precipitate, as also the gelatin and hide powder residues, is stained very dark brown by the reagent.

The method proves very convenient in studying the distribution of tannin in fruits and other parts of plants. Tissues which can be distinguished only with difficulty in the unstained hand section become very sharply differentiated after treatment with nitrous ether vapor. For example, a piece of the woody fruit stem of the date stained in this way reveals a very narrow tannin zone near the cuticle, corresponding very closely to the more prominent one in the fruit. In thin section this zone resolves into rather widely separated, very small, dark-colored cells. A large Japanese persimmon weighing one-half pound can be completely stained in 24 to 48 hours without any abrasion of the cuticle. The process offers the further advantage that the tannin is deposited in the cells where it occurs, and thus eliminates all danger of carrying soluble tannin across the face of a section. In the date the outer tannin-bearing tissue detaches and may be separated very easily and sharply from the inner tannin-free tissue after prolonged exposure to ethyl nitrite. In some fruits, certain cells stain violet or lilac instead of brown, while others take no stain whatever until after prolonged treatment. This is notably true in the wild persimmon, which stains brown very readily just beneath the skin and again at the center of the fruit, with the exception of occasional cells interspersed through the flesh. Further exposure to the vapor of nitrous ether stains all the tannin cells uniformly brown. It is not evident whether this phenomenon depends entirely on permeability of the cell walls, or whether there is an actual difference in chemical composition of the cell contents.

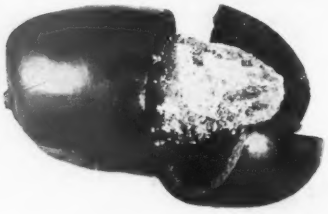


FIG. 8.—Deglet Noor date showing sharp separation of tannin layer from inner flesh after ethyl nitrite treatment.

For laboratory use a 20 per cent. alcoholic solution made by diluting the 90 per cent. commercial nitrous ether is recommended. Amyl nitrite may be used, but is disagreeable to work with. Ordinary sweet spirits of niter, which contain about 4 per cent. ethyl nitrite, may be used, but will require much longer exposure.—A. E. VINSON, *Arizona Agric. Exper. Station, Tucson, Arizona.*

CURRENT LITERATURE

BOOK REVIEWS

Phylogeny of plants

The first volume of LOTSY's huge work, dealing with the thallophytes, was published in 1907 and was noticed in this journal.[1] The second volume has now appeared[2] and includes the "archegoniates." The speed at which such large volumes have been organized for publication, and the extensive range of literature traversed in compiling the facts and the illustrations, are both impressive. But no one man can deal authoritatively with the whole plant kingdom, and the wisdom of so comprehensive a plan by one author is questionable. If it be undertaken, the usefulness of the work depends upon his appreciation of the relative values of investigations, his general perspective, and his organizing power. LOTSY has stood this test as well as could be expected, and although there are abundant grounds for criticism, the book is on the whole a fairly logical and copiously illustrated digest of the current literature. It will serve as a useful encyclopaedia of general information in reference to the present knowledge of bryophytes, pteridophytes, and gymnosperms, and the current speculations regarding them. Of course the whole thesis is the evolution of the plant kingdom.

The chief criticism must fall undoubtedly on the sometimes radical changes proposed in classification on debatable grounds, and on the author's conceptions of phylogeny, which others may be disposed to consider erratic. Thus, in the bryophytes, Anthoceros is accorded the status of the most primitive liverwort, on the ground that it is nearest the algae in having in each cell only one chloroplast and this furnished with a pyrenoid. Then, having discussed Riccia as the liverwort with the simplest sporophyte and Sphaerocarpus as "the simplest known liverwort," LOTSY predicates a "Sphaeroriccia" as the hypothetic *Urform*, from which Anthocerotales, Marchantiales, and "Metzgeriales" separately sprang, the latter spraying out into the Jungermanniales (Acrogynae).

The portion of the volume dealing with pteridophytes (318 pp.) and the "polyciliate" gymnosperms (80 pp.) gives opportunity to add to the older data an unusually large volume of recent work, and LOTSY does not hesitate to formu late here also his own theories, even in connection with groups in which he has done no investigation. The following statements may be taken as illustrations:

[1] BOT. GAZETTE **43**:421. 1907.

[2] LOTSY, J. P., Vorträge über botanische Stammesgeschichte, gehalten an der Reichsuniversität zu Leiden. Ein Lehrbuch der Pflanzensystematik. Zweiter Band: Cormophyta Zoidogamia. Imp. 8vo. pp. 902. *figs. 553*. Jena: Gustav Fischer. 1909. *M* 20.

"We can think of Marattia as having come from Angiopteris by the fusion of sporangia"; "the fertile spike of Botrychium is not a whole leaf, but only a branch of the foliage leaf"; "Marattiales and Cycadofilices are two branches differentiated from Primofilices."

The taxonomic groupings in certain particulars seem to be more original than necessary. The Sphaerocarpaceae, including as a tribe the Rielleae, appear among the Marchantiales; the Jungermanniales Anacrogynae have become the Metzgeriales, leaving the Acrogynae only as the Jungermanniales; and many novel arrangements appear in the Bryales, which cannot be stated in detail. Of course the vascular groups presented are all "Diploidales," and the two principal divisions are the *Biciliaten* and the *Polyciliaten*. This naturally results not only in detaching Isoetes from the Lycopodiales, but also subordinates the boundary between pteridophytes and spermatophytes. There may be no objection to either of these results, but the single character used to secure them is more than questionable.

The bibliography is copious, but by the method of its presentation it is repetitious and not specific; nor is it so complete in its citations as one would expect from the editor of a bibliographical journal. The index is unfortunately double, but otherwise excellent, and puts the reader in easy command of the contents.—J. M. C. and C. R. B.

MINOR NOTICES

A flora of the Dutch West Indies.[3]—The first volume of this work deals with the flora of the islands of St. Eustatius, Saba, and St. Martin. The work is divided into three parts. The first part is concerned with taxonomy, the second presents a historical account of the flora, and the third deals with the phytogeography of the islands. The taxonomic portion is based mainly on the collections of SURINGAR, VAN GROL-MEYERS, LIONARONS, and BOLDINGH, whose collections are deposited either in the Rijks herbarium at Leiden, the herbarium at Utrecht, the Colonial Museum at Haarlem, or in the herbarium of KRUG and URBAN at Berlin. The sequence of the families and genera is essentially that of DE DALLA TORRE and HARMS'S *Genera Siphonogamarum*, and the nomenclature is in accordance with the International Rules adopted at Vienna in 1905. The taxonomy occupies the major part of the volume, covering over 200 pages, and consists of an enumeration of the species with reference to original publications, a very limited synonomy, and the citation of plants collected on the different islands. About 500 genera and approximately 800 species are recorded. The second part contains interesting and important historical matter, particularly as to itineraries of collectors and lists of their plants. The third part embraces detailed information as to the natural features of the islands, a series of tables on distribution, a bibliography, and finally an index to vernacular and scientific

[3] BOLDINGH, I., The flora of the Dutch West Indian Islands St. Eustatius, Saba, and St. Martin. Roy. 8vo. pp. xii + 321. *maps* 3. Leiden: E. J. Brill. 1909.

names. The volume is of moderate size and well printed, but the introduction of keys, especially in case of some of the larger genera, would have added somewhat to the usefulness of the work in the identification of species. Nevertheless, as a catalogue of the known species of the islands, it is a commendable contribution to taxonomic literature and will serve as an excellent basis for future studies on the flora of the region.—J. M. GREENMAN.

Natürlichen Pflanzenfamilien.[4]—Parts 236 and 237 contain a supplement to the Conjugatae and Chlorophyceae by the distinguished algologist Professor N. WILLE. Two new genera are included, namely *Pseudopringsheimia* and *Pseudulvella* of the Chaetophoraceae; the former is based on *Ulvella confluens* Rosenv., an epiphyte growing on marine algae of the north Atlantic; the latter is based on *Ulvella americana* Snow, also an epiphyte, and occurs on freshwater algae of North America. Parts 238–240, likewise of recent issue, consist of a general index to Teil I of this great work.—J. M. GREENMAN.

NOTES FOR STUDENTS

The life history of Griffithsia.—LEWIS[5] has published a paper on the life history of *Griffithsia Bornetiana*. The paper begins with the presentation of the vegetative characters of the three different individuals of this species: antheridial, procarpic, and tetrasporic. The author records comparative studies of the size of the cells in the three individuals, discusses the nature of intercellular connections, describes the division of chromatophores, and calls attention to the phenomenon of an approximate simultaneous occurrence of the nuclear divisions in multinucleate cells. There are then described the development of antheridia, cystocarps, and tetraspores; the methods of vegetative propagation; germination of spores; and tetraspore-like structures on sexual plants. The paper closes with a discussion of results, in which the author presents his views on alternation of generations in Griffithsia.

Some of the cytological results are as follows. The nuclei in Griffithsia are throughout their history very poor in linin. The chromatin of the resting nucleus, therefore, is not distributed on a linin reticulum, but is chiefly contained in a centrally placed, homogeneous nucleolus or "karyosome." As regards the mode of formation of chromosomes, the chromatin passes out of the nucleolus until the whole chromatin content is distributed through the nuclear cavity in the form of granules. These granules are much more numerous than the chromosomes, and probably by the fusion of separate granules chromosomes are formed. The number of chromosomes in the nucleus of vegetative cells in the tetrasporic plant

[4] ENGLER UND PRANTL, Die natürlichen Pflanzenfamilien, etc. 236 and 237 Lieferung. Conjugatae und Chlorophyceae von N. WILLE. Nachträge zum I. Teil, 2 Abt., Bogen 1–6, pp. 96; 238 bis 240 Lieferung. Register zu Teil I (vollständig), pp. 242. Leipzig: Wilhelm Engelmann. 1909. *M* 3, *M* 4.50.

[5] LEWIS, I. F., The life history of *Griffithsia Bornetiana*. Annals of Botany **23**:639–690. *pls. 49–53*. 1909.

seems to be with certainty fourteen, and that of the sexual plant is about seven. Spindle fibers are formed, apparently by the rearrangement of the linin thread, so that the spindle is intranuclear. Kinoplasmic caps are present only during the nuclear division. The changes in the nucleus of the tetraspore mother cells are striking. The nucleolus fragments into several rounded bodies of various sizes, which after continuous fragmentation yield 12–14 rounded masses of chromatin of about the same size. These bodies become again irregular in form, and fuse with one another, so that their number is reduced by more than half. This stage LEWIS considers to represent synapsis, but it differs from the usual type. Fourteen chromosomes then appear and are scattered in the nuclear cavity. After telophase of the first division in the tetraspore mother cell, the daughter nuclei rest before commencing the second division. In the sporelings from tetraspores about six or seven chromosomes appear; in those from carpospores the number has not been ascertained exactly, but the author believes them to be diploid. Cell division in sporelings occurs usually when about sixteen nuclei are present, so that the coenocytic condition is attained very early in Griffithsia. Tetraspore-like structures on an antheridial plant have been found in only one case out of all plants examined. In this structure the cleavage furrow either does not reach the center of the cell or no trace of it occurs. Nuclear conditions have not yet been followed thoroughly.

From the cytological evidence brought forth in this paper, LEWIS considers that there exists in *Griffithsia Bornetiana* an alternation of generations similar to that described for *Polysiphonia violacea* by the reviewer. The fusion nucleus, which contains fourteen chromosomes, produces the cystocarp in which are formed carpospores. The nuclei of tetrasporic plants contain fourteen chromosomes, and it seems reasonable therefore to assume that the tetrasporic plants arise from carpospores. In tetraspore formation the number is reduced one-half, and seven chromosomes enter the nucleus of the tetraspore. It seems probable that on germination the tetraspore gives rise to the individual whose nuclei contain seven chromosomes and which bears sexual organs.

As to whether the alternation of generations in Griffithsia is to be regarded as antithetic or homologous, the conclusions are as follows: (1) "There is in Griffithsia an antithetic alternation of generations, the gametophyte being represented by the sexual plants, the sporophyte by the sporogenous cells of the cystocarp." (2) "In addition to this there is a regular succession of tetrasporic individuals and sexual individuals. The tetrasporic individuals resemble the sporophyte in the number of chromosomes; they resemble the gametophyte in morphological differentiation. They are to be considered as a phase of an homologous alternation of generations, not the equivalent, wholly or in part, of the sporophytes of archegoniates." To draw these conclusions, LEWIS has put more weight upon the two following factors than upon the fundamental chromosome difference between sporophytic and gametophytic nuclei: (1) the outer morphological similarity of the tetrasporic plant to the sexual plant; (2) the fact that either seems capable of producing the outer morphological equivalent of the reproductive

structures of the other, although the real nature of the structures has not yet been determined.—SHIGÉO YAMANOUCHI.

Development and biology of Armillaria.—FISCHER,[6] of the Indian Forest Service, has given a brief but interesting account of the development of the fruit bodies of *Armillaria mucida* Schräd., an agaric with a very slimy outer surface, which is common in parts of Europe, growing often in dense tufts on certain of the hard-wood trees. There is a thin universal veil present from the primordium stage to the time that the fruit body is rapidly expanding, which consists of interwoven threads forming a layer two or three cells deep. In an early stage of the primordium a palisade layer of cells is formed over its convex upper surface, just underneath the universal veil. This marks off the pileus, which now begins to expand laterally, also loosening the fundamental tissue between its lower margin and the future stipe, thus providing for the gill cavity, while at the same time the development of a palisade layer is continued from the margin inward over the roof of the gill cavity to form the primordium of the hymenium. The loose fundamental tissue between the margin of the pileus and the outer surface of the stem forms the partial veil. The slime which covers the plant is formed from the mucilaginization of the outer ends of the palisade tissue on the surface of the pileus.

The writer states (p. 504) that the present reviewer "seems to accept HARTIG'S account of the development in *Armillaria mellea* as substantially correct." Thus is discretion in the matter of not prejudging a case which is under investigation rewarded! A study of the development of *Armillaria mellea* was made by the reviewer several years ago, and an account[7] of it was presented before Section G of the A. A. A. S. at the New Orleans meeting, in connection with that on *Agaricus campestris*, the latter of which was published.[8] He has been holding the work on *Armillaria mellea* for some further study to clear up some details. There is nothing in this paper on *Agaricus campestris* which can be construed as either supporting or contradicting HARTIG'S account, and the writer carefully held to neutral ground.

It is to be hoped that FISCHER will continue his studies in the Agaricaceae, and that others also may be induced to undertake similar work. But it is just as much to be desired that either good photomicrographs be used to illustrate the work, or that good drawings be made, for little praise can be given to the illustrations accompanying this otherwise creditable paper.—GEO. F. ATKINSON.

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[6] FISCHER, C. C. E., On the development of the fructification of *Armillaria mucida* Schräd. Annals of Botany **23**:503–507. *pl. 35. figs. 1–7.* 1909.

[7] ATKINSON, GEO. F., The development of *Armillaria mellea;* the development of *Agaricus campestris.* Proc. A. A. A. S. 53rd Meeting, Dec. 1905—Jan. 1906. *Ibid.* Science N. S. **23**:203. 1906.

[8] ———, The development of *Agaricus campestris.* BOT. GAZETTE **43**:215–221. *pls. 7–12.* 1906

FISCHER has also investigated the biology of *Armillaria mucida*,[9] more especially with a view to determining whether the fungus is a parasite or a saprophyte. Most writers simply state that the fungus grows on beech trees, but MASSEE records that "at High Beech, Epping Forest, ... a healthy branch of a beech having been broken off, the wound was inoculated with the spores of *A. mucida*. At the end of the second season after the inoculation the branch was killed for a considerable distance, and the sporophores of the fungus appeared in abundance." FISCHER, unwilling to accept this observation as proof that the fungus is a parasite, has attempted to infect wounds made in living beech trees with spores or with mycelium. His experiments gave negative results, so that he was unable to obtain any proof of the alleged parasitism of the fungus. On the other hand, he found that the fungus could be grown readily as a saprophyte on various substrata, such as bread, dead beech wood and twigs, and also upon gelatin containing beerwort, meat extract, or malt extract. The time elapsing between the sowing of the spores and the ripening of the fruit bodies in pure cultures varied from 51 to 109 days. The spores germinate readily in water as well as in various culture media. FISCHER'S inoculations were carried out on thin branches. Possibly, if stouter branches containing older wood had been used, positive results might have been obtained. So far, however, there does not seem to be any clear evidence that the mycelium of *A. mucida* can kill the living parenchyma and medullary ray cells in beech wood.

FISCHER states that a spore, after arising somewhat laterally on its sterigma, "only assumes the central position later on as it approaches maturity." The figure given of the mature basidium does not support the statement that the spore is situated symmetrically over the sterigma. If FISCHER'S observation is correct, then *Armillaria mucida* is a marked exception to the general rule for the position of the mature spores in Hymenomycetes. There is one misquotation, doubtless due to a printer's error, from a paper by the writer. The number of spores that I found to have been produced from a large fruit body of *Polyporus squamosus* was 11,112,500,000, and not 11,112,500 as stated.[10] FISCHER urges that since the number of spores produced from a fruit body is so vast, wounds on trees must often become infected, and that stumps or timber infected with *Armillaria mucida*, as well as its fruit bodies, should be destroyed when possible. It may be added that eleven thousand million spores would be sufficient to provide one for each square inch in nearly three square miles of level ground.—A. H. REGINALD BULLER.

Chemotropism of pollen tubes.—In 1889 MOLISCH showed that pollen tubes grow toward pieces of the stigma (chemotropism), and grow away from the edge of a cover-glass preparation (aerotropism). Five years later MIYOSHI found that

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[9] FISCHER, C. C. E., The biology of *Armillaria mucida* Schräder. Annals of Botany **23**:515–535. *pls. 36, 37.* 1909.

[10] BULLER, A. H. R., The biology of *Polyporus squamosus* Huds., a timber-destroying fungus. Jour. Econ. Biol. **1**:114. *fig. 6.* 1906; also Researches on Fungi. Part I, chap. 5, 1909. London: Longmans, Green & Co.

various carbohydrates deflected pollen tubes, and in 1899 LIDFORSS reported that they were responsive also to certain proteins. In the last ten years, with the exception of a few papers by the same author, nothing has appeared on the responses of pollen tubes. Now LIDFORSS, who has been prosecuting his researches year after year, in the short periods available each season for any particular plant, presents the results in detail.[11] First he discusses the effect of various chemical agents upon the germination of the pollen. In the section on proteo-chemotropism, LIDFORSS lists the large number of proteins of various groups that have yielded definite results. In general it may be seen that the pollen tubes are indifferent to cleavage products of proteins, and are harmed by the albumoses and peptones tried. On the contrary, to the albumins, globulins, nucleo-albumins, coagulated proteins, and glycoproteins, nineteen in all, the tubes respond by positive curvatures. The nucleo-proteins differ much, some being attractive, some apparently indifferent, and some poisonous. Besides the tropic action of the proteins, they accelerate the growth of the tubes, which also tend to branch in some cases. The reaction time in most favorable cases for *Vallota purpurea* is less than 5 min., and for *Narcissus Tazetta* and *Tradescentia virginica* less than 2–3 min. The liminal value of the concentration was difficult to determine. In the case of diastase with *Vallota purpurea* it was about 0.1 per cent. Only one case of apochemotropism with proteins was observed (*Scilla campanulata* with diastase), and as this could not be confirmed by later experiments it was possibly due to some impurity of the diastase used. Proteo-chemotropism has been found in 13 families of monocotyls and 42 families of dicotyls, so that it may be considered as of general occurrence in angiosperms.

Saccharo-chemotropism was not investigated extensively by LIDFORSS, but his limited observations, combined with those of MIYOSHI, make it probable that responses to various carbohydrates are also general among angiosperms. Osmotropism was clearly shown by some tubes, and the author promises a later paper on this subject. In a special part, an appendix to the body of the paper, there are details regarding all the species of pollen studied, as to favorable conditions for germination and the responses obtained. These furnish useful details for laboratory directions in employing these experiments in instruction.—C. R. B.

Persistence of characters in Aspergillus.—KOMINAMI[12] has investigated the persistence of characteristics induced in *Aspergillus niger* by subjecting that mold to unusual cultural conditions, in this case strong solutions of common salt. The conidia used in the experiments were obtained from three strains: (1) from cultures on normal nutrient media; (2) from cultures grown for one generation on nutrient solution containing 6 per cent. of salt; and (3) from cultures grown for

[11] LIDFORSS, B., Untersuchungen über die Reizbewegungen der Pollenschläuche. I. Der Chemotropismus. Zeit. Bot. **1**:443–496. *pl. 3.* 1909.

[12] KOMINAMI, K., Biologisch-physiologische Untersuchungen über Schimmelpilze. Jour. Coll. Sci. Tokyo **27**:1–33. *pls. 3.* 1909.

ten generations on media containing 5 or 6 per cent. of salt. Conidia from each of these strains were sown in solutions containing 20.4 to 22 per cent. of salt. It was found that conidia from strains which had previously been accustomed to 6 per cent. salt solution germinated more rapidly and grew more vigorously than conidia from normal solutions. Conidia from the strain accustomed for ten generations to salt showed the same effect in a more pronounced manner. When conidia from all strains were sown on normal nutrient solution, the strains adapted to salt grew more poorly and consequently fruited sooner than the strains not adapted. When all strains were sown on salt solution, the reverse effect was observed. The accommodation to salt solution did not disappear after ten generations of culture on normal media. Attempts to establish persistency of accommodation to higher osmotic pressures and to some poisons gave negative results.

The author has attacked this problem, as he states in the introduction, from the standpoint of inheritance of acquired characters. It seems doubtful if the idea of inheritance can be applied perfectly in such cases as described in the foregoing, where the whole protoplasm in the organism is modified by a factor in the environment, and a part of this modified protoplasm passes into the conidium—an asexually formed bud of the original stem. So long as no sexual process intervenes, the succeeding "generations" must be looked upon as a continuation of the original plant. Therefore, organisms with only asexual reproduction are not well suited to the study of the inheritance of acquired characters. That some of the characteristics should not be lost readily, i. e., that the organism should not readapt itself readily to the former condition, may appear unusual, but it is possible that the reactions by virtue of which the accommodation occurs are not readily reversible. If this principle were general, it would lead to the existence of numerous physiological races. Such races are common among parasitic fungi, and it may be that they are even more numerous and as highly specialized among the saprophytes.—H. HASSELBRING.

Tubercle bacteria.—DE'ROSSI working at Perugia has been investigating anew the *Bacillus radicicola* of BEIJERINCK, and has reached results so at variance with those of previous observers as to need ample confirmation.[13] His conclusions may be stated briefly as follows:

An examination of the literature convinces him that many observers have mistaken *germi banali* of the soils for the real *B. radicicola;* and others have worked with impure cultures of it. The certain isolation of the true germ is attained by spreading the contents of a tubercle on a plate of gelatin with a leguminous extract containing glucose, and rejecting the colonies which develop rapidly, in favor of those which become visible to the microscope on the fifth or sixth day and to the eye on the tenth to twelfth, the form really desired being apparently a contaminant. These show the true morphological, cultural, and biological characters of *B. radicicola.*

[13] DE'ROSSI, G., Studi sul microorganismo produttore dei tubercoli delle leguminose. Annali di Botanica **7**:617–669. *pl. 23.* 1909.

Of the various preparations proposed for the inoculation of fields, the dried cultures of MOORE and of BOTTOMLEY are absolutely useless; it is certain that they either do not contain the tubercle organism at all, or it is in such condition as to be unable to show its action in any way. As to the efficacy of fresh cultures (like the nitragine of HILTNER) opinions are not unanimous, and one cause of the uncertainty of the results may be that the culture is eventually impure.

In soils without tubercle bacteria those properly isolated by DE'ROSSI from the same species of host have given him good results in increased crops and added proportion of nitrogenous content. The manner of infection has had no effect, and he has not been able to discover any such unfavorable influence as was attributed by HILTNER and STORMER to substances in the soil or on the seed at the time of germination. From negative results DE'ROSSI doubts the possibility (affirmed by some) of improving the crop by inoculation when the soil already contains the organism. In another paper of the same title (included in above citation) DE'ROSSI asserts that in his cultures, certainly pure and surely identified as the tubercle bacillus, no fixation of free nitrogen has taken place. As this is not observed in the tubercle until the bacteroids are formed, and as few bacteroids are formed in cultures, the result appears quite proper. Evidently the questions raised will require new examination.—C. R. B.

Evolution of fungi.—The construction of a phylogenetic system for the fungi has always been peculiarly difficult on account of the heterogeneity of the group and the lack of common characters uniting the forms into evolutionary series. Two theories have generally been suggested; one regarding the fungi as an autonomous group, and the other regarding them as offshoots from various groups of algae. Among the adherents of the algal theory of descent, a favorite method of treating the lower Phycomycetes (or Chytridiales) has been to consider them as forms derived from the higher Phycomycetes through the degenerating influence of parasitism.

ATKINSON[14] in dealing with the evolution of this group favors the view that the Phycomycetes constitute an ascending evolutionary series. In support of this view he points out that the favorite method of accounting for the lower forms is fallacious, for whatever evolution occurred among the fungi took place after they had acquired a parasitic or saprophytic mode of life. Parasitism and saprophytism, therefore, were general modes of life under which the whole group existed, and hence cannot be invoked as special factors to account for particular minor offshoots. Another argument for the unity of the group is found in the phenomenon of diplanetism, that is, the occurrence of two swarming periods of the zoospores. Although this phenomenon is most clearly developed in the Saprolegniales, the author believes it occurs in a primitive state in the Chytridiales, in some of which the protoplasm breaks up into the sporangium, where after an interval the zoo-

[14] ATKINSON, G. F., Some problems in the evolution of the lower fungi. Annal. Mycol. **7**:441–472. *figs. 20.* 1909.

spores are differentiated and set free. The first partial differentiation and movement of the spore-origins is regarded as the counterpart of the first swarming period of the zoospores of the Saprolegniales. This view derives diplanetism from a more primitive state among the lower fungi, while there is nothing among the algae which corresponds to the phenomenon.

It is further pointed out that some other phenomenon, such as heterogamy, isogamy, and the proliferation of the sporangium, each have a primitive counterpart among the Chytridiales, and can be traced in a natural series to the Saprolegniales and Oomycetes. For some of the phenomena, like the proliferation of the sporangium, there is no counterpart whatever among the algae.—H. HASSELBRING.

Cultures of Uredineae.—In continuation of the long series of experiments in his cultural work on the Uredineae, the cultures made in 1908 have been reported by ARTHUR.[15] The most interesting result of the work of that year is the discovery that the aecidial stage of *Gymnosporangium externum* Arthur and Kern (described as new) occurs on a herbaceous perennial, *Porteranthus stipulatus* (Muhl.) Britton. This is the only case known of the occurrence of an aecidium of Gymnosporangium on a plant outside of the Pomaceae. Another unusual case is that of *G. Libocedri* (P. Henn.) Kern, whose aecidial stage is shown to be *Aecidium Blasdaleanum* D. and H., a true aecidium and not belonging to the Roestelia type as in all other Gymnosporangia. Series of cultures with 13 species of rusts gave negative results, and cultures with 23 species confirmed and supplemented work previously reported. The relations of the following species have been worked out for the first time: *Puccinia absinthii* DC. on *Artemisia dracunculoides* Pursh has no aecidia, but has pycnidia on the same host; *P. macrospora* (Peck) Arthur on *Carex comosa* Boott sown on *Smilax hispida* Muhl.; *P. patruclis* Arth. on *Carex pratensis* Dreej. sown on *Agoseris glauca* (Pursh) Green; *P. cinerea* Arth. on *Puccinellia airoides* (Nutt.) Wats. and Coult. sown on *Oxygraphis cymbalaria* (Pursh) Prantl; *P. Koeleriae* Arth. on *Koeleria cristata* (L.) Pers. sown on *Mahonia aquifolium* (Pursh) Nutt.; *P. alternans* Arth. on *Bromus Porteri* (Coult.) Nash sown on *Thalictrum dioicum* L.; *P. obliterata* Arth. on *Agropyron biflorum* R. and S. sown on *Aquilegia canadensis* L.; *P. Muhlenbergiae* Arth. and Holw. on *Muhlenbergia glomerata* Trin. sown on *Callirhoe involucrata* (T. and G.) A. Gray; *Gymnosporangium Libocedri* (P. Henn.) Kern on *Libocedrus decurrens* Torr. sown on *Crataegus Pringlei* Sarg.; and *G. externum* Arth. and Kern on *Juniperus virginiana* L. sown on *Porteranthus stipulatus* (Muhl.) Britt.—H. HASSELBRING.

Biology of Gymnosporangium.—Another interesting example showing the slight degree of differentiation existing among some species of parasitic fungi

[15] ARTHUR, J. C., Cultures of Uredineae in 1908. Mycologia 1:225–256. 1909.

has been brought out by FISCHER[16] in the case of the gymnosporangia inhabiting Juniperus. A casual attempt to infect *Sorbus aria*, *S. aucuparia*, and some other species, with teleutospores obtained from *Juniperus communis* and supposed to be those of *Gymnosporangium tremelloides*, resulted in no infections. This experiment showed that the teleutospores were neither those of *G. tremelloides* nor those of *G. juniperinum*, whose aecidial host is *Sorbus aucuparia*. These results led to a series of cross infections, in which the teleutospores of the Gymnosporangium were used to infect a number of pomaceous plants, and aecidial spores from these plants were sown on Juniperus. The work showed that two forms of Gymnosporangium occur on *Juniperus communis* and *J. nana*. The first form occurs chiefly on the stems and branches, rarely on the leaves, and has its aecidial form on *Amelanchier ovalis*. The first aecidia appear on *A. ovalis* 55 to 60 days after the sowing of teleutospores, and the teleutospores appear on Juniperus about 20 months after the sowing of aecidiospores. For this form the specific name *Amelanchieris*, originally applied to the Roestelia on Amelanchier, is reserved. The second form occurs chiefly on the leaves of Juniperus, and has its aecidial form on *Sorbus aucuparia*, but does not infect *A. ovalis*. The aecidia appear 45 to 50 days after the sowing of teleutospores, and teleutospores are produced in the spring following the sowing of the aecidiospores in late summer. This is the form known as *G. juniperinum*.

For a discussion of the history, nomenclature, and geographical distribution of the two forms, as well as of their respective hosts, the reader is referred to the original paper.—H. HASSELBRING.

Fermentation of tobacco.—Theories relating to the fermentation of tobacco have been based either on the view that the process is due to bacterial action, or to the action of enzymes without the intervention of bacteria. One investigator alone, SCHLOESING, seems to have believed that the later part of the fermentation is due to purely chemical oxidations. In view of these conflicting theories describing the process, the experiments of BOEKHOUT and DEVRIES are especially interesting.[17] These authors attacked the problem by methods similar to those used in their recent work on the spontaneous heating of hay. Dry samples of cured tobacco were sealed up in tubes with oxygen or air, and in some cases water was added to the samples. The tubes were then heated to 100° or to 33°, according to the purpose of the experiment, and after a definite period of heating the gas in the tubes was analyzed. It was found that oxidation took place at both tempera tures, but more rapidly at the higher temperature. The presence of water increased the rate of oxidation. By the process of oxidation carbon dioxid was liberated and oxygen was fixed. It was found that starch and pentosans were

[16] FISCHER, ED., Studien zur Biologie von *Gymnosporangium juniperinum*. Zeit. Bot. **1**:683–714. *figs. 8*. 1909.

[17] BOEKHOUT, F. W., AND DEVRIES, O. J. J., Ueber Tabaksfermentation. Centralbl. Bakt. II. **24**:496–511. 1909.

the principal substances which disappeared. In seeking for a catalyzer, iron and manganese were considered. It was found that starch paste with traces of ferrosulfate was completely hydrolyzed in six days at 100°. In a similar experiment without iron salts no hydrolysis took place.

The authors conclude from their work that the fermentation of tobacco is a process of oxidation, in which iron salts may act as catalyzers. They believe that it is almost certain that the processes are of a purely chemical nature, that is, take place without the action of enzymes or bacteria.—H. HASSELBRING.

Ascent of water.—DIXON recounts[18] some experiments intended to show that the living cells of the wood do not influence the rate at which water is transmitted through a stem. Arguing that if there is any sort of action which even facilitates the passage of water upward, its effect would be noticeable experimentally by a downward filtration of water more rapid in a killed stem than in a living one, he arranged two like shoots of syringa so that he could keep the two at the same temperature and could determine the amount of water that would pass downward through them, both being alive, under a given head of pressure in a short time, say 10 minutes. One shoot was then killed, by steam or by poison, with no disturbance, and the amount of water transmitted by both again determined. No appreciable or constant difference was found; whence DIXON argues that vital action is at least unlikely.

On the basis of the strain borne by the water of a soap-bubble film, DIXON calculates that the tensile strength of water, even when saturated with air, is not less than 42.5 *A*, a figure which agrees fairly well with BERTHELOT'S early determination, 50 *A*, to which he calls attention. In another paper,[19] DIXON presents some further experimental work on this point. He finds a tensile strength under certain conditions of more than 150 *A*. There is no longer doubt that the cohesion of water is sufficient to stand the strain involved in a lift; but what are the resistances to be overcome? EWART says about 50 *A;* DIXON thinks this too high, and suggests a maximum of 20 *A* for the tallest trees (100^{m}). In the absence of determinations of the osmotic pressure in the leaves of such trees, DIXON thinks it fair to assume that it is as much as 20 *A*. Others will deny the assumption. And, as Mr. Dooley pointedly says, "There y' are!"—C. R. B.

Life of pollen.—PFUNDT,[20] working under the guidance of PFEFFER, has determined the viability of pollen in air of various degrees of humidity. Incidentally he presents many data regarding the germination of pollen that will be useful.

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[18] DIXON, H. H., Vitality and the transmission of water through the stems of plants. Notes from the Botany School of Trinity College **2**:5–18. 1909.

[19] ———, Note on the tensile strength of water. *Ibid.* 38–43. Both "Reprinted from Proc. Roy. Dublin Soc.," without citation of volume or pages.

[20] PFUNDT, MAX, Der Einfluss der Luftfeuchtigkeit auf die Lebensdauer der Blüthenstaubes. Jahrb. Wiss. Bot. **47**:1–40. 1909.

It is found that the duration of life depends directly upon the humidity of the air, being greatest at one vapor pressure and least at another; but these points do not coincide for all kinds of pollen. There are only a few species which live longest in very moist air (90–60 per cent.), and only one that lives the same length of time in moist as in dry air; but there are many species which live longest in very dry air (30–0 per cent.). (A priori, it would seem that all pollen should remain alive longest in absolutely dry air, and one wonders whether the differences found by PFUNDT are not due to some other factors, for whose operation there is ample room in the handling of the material, and particularly in the artificial germination tests on which the conclusions are based.) In nature the duration of life is very variable because of the variations in the moisture of the air. The influence of a single change from moist to dry is not very evident, but repeated changes shorten life, and the drying of wet pollen leads quickly to death, the sooner the longer it was wet. Ecological adaptations are not clear; the pollen of early spring and late autumn flowers, however, is generally long-lived, shows little sensitiveness to moisture, and germinates at minimum temperatures, even below 4–5°. Whereas freshly gathered pollen may germinate in dilute or in concentrated solutions indifferently, that which is about to die produces tubes only in the most favorable concentration.—C. R. B.

Morphology of Salvinia.—ARNOLDI[21] has published the results of a study of *Salvinia natans*. The paper is divided into three parts: (1) the germination of the microspore and the development of the male gametophyte; (2) the germination of the megaspore and the development of the female gametophyte, fertilization, and embryo formation; (3) a series of experiments on the female gametophyte. In the first part little is added to BELAJEFF'S account except such cytological details as the size of nuclei and chromosomes, the number of chromosomes (4), and the spermatogenesis. The early stages of the female gametophyte were followed more closely than by previous authors. It is found to be composed of a coenocytic portion contained within the old spore wall, and an exposed chlorophyll-bearing tissue, one side of which bears archegonia. In the mature archegonium, the axial row consists of the egg, the ventral canal cell, and a broad wedge-shaped neck canal cell which is binucleate. In the third part of the paper the following facts are recorded: (1) an unsuccessful attempt to produce apogamous embryos: (2) the appearance of the archegonia in the upper or lighter side of the prothallium is not a response to light; (3) the development of the winglike appendages, characteristic of the female gametophyte of Salvinia, occur only when fertilization and embryo formation precede it; (4) the food material used by the developing embryos is not made by the chlorophyllose tissue of the gametophyte, but is that which was stored in the megaspore; (5) spores sown on damp clay germinated and produced good prothallia with normal archegonia and embryos.—WANDA M. PFEIFFER.

[21] ARNOLDI, W., Beiträge zur Morphologie der Keimung von *Salvinia natans*. Flora **100**:121–139. *figs.* 47. 1909.

Geotropism.—GROTTIÄN[22] finds that by the use of proper concentrations of amyl alcohol and other anesthetics it is possible to stop geotropic response in horizontally placed roots without entirely stopping growth. He believes he has shown that anesthetics may abrogate the power of perception without entirely eliminating the power of reaction. He finds that certain concentrations of the anesthetics accelerate growth, as has been shown by several other investigators. He finds likewise much evidence, though not entirely conclusive, that concentrations which greatly stimulate growth also shorten reaction time.

The same author has repeated all of CZAPEK's experiments on the change of metabolism due to geotropic stimulation. As is well known, CZAPEK claims that geotropically stimulated root tips show a greater percentage of homogentisic acid than unstimulated ones, and that the accumulation is due to the development of an antienzyme which stops the action of the enzyme which carries on the further metabolism of the acid. Several authors have already shown that CZAPEK's method of detecting the acid are not at all reliable, and even that neither tyrosin from which the acid is derived nor the acid itself is present in the root (geotropically stimulated or not) in detectible quantities. GROTTIÄN finds that even CZAPEK's inadequate methods do not give, on the average, any difference between stimulated and unstimulated roots. It seems then that CZAPEK's methods do not indicate any change in the metabolic products due to geotropic stimulation, much less the accumulation of homogentisic acid.—WILLIAM CROCKER.

In 1908 BLAAUW determined that in stimulation by light the presentation time is inversely proportional to the intensity of the light used. The suggestion that the same would be found true with the effective force in stimulation by gravitational and centrifugal acceleration has been tested by Miss C. J. PEKELHARING, who reports[23] that she has established this relation for the same plant under identical conditions with continuous stimulation. This completes the proof that with both sorts of stimuli and with both continuous and intermittent application, the product of the presentation time and the force of the stimulus is a constant. The validity of WEBER's law for geotropic curvatures is questioned, and FITTING's experiments on this point are considered inconclusive. By testing the action of gravity and light together, it appeared that the mode of perception of the two is not the same. There was not the least evidence that positive or negative reaction to gravity could be obtained by increasing or decreasing the strength of the stimulus. One of the most striking results of the investigation has been emphasized by WENT (under whose direction it was conducted) in a separate

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[22] GROTTIÄN, WALTER, Beiträge zur Kenntniss des Geotropism. Beih. Bot. Centralbl. **24**:255–285. 1909.

[23] PEKELHARING, CATHARINA J., Onderzoekingen over die perceptie van den zwaartekrachtprikkel door planten. Proefschrift ter verkrijging van der graad Doctor in die plant- en dierkunde, aan de Rijks-Universiteit te Utrecht. 8vo. pp. 105. *pls. 4*. 1909.

communication to the Amsterdam Academy.[24] Miss PEKELHARING found it possible, by using potash-alum properly balanced in the culture solution, to grow some roots of *Lepidium sativum* fairly straight and free of starch grains. Nevertheless, these roots, in many cases, showed geotropic curvatures; from which it is evident that there is perception independent of statolith starch, however useful this may be when present.—C. R. B.

Chromosomes of Taraxacum and Rosa.—In 1903 RAUNKIAER found that Taraxacum developed embryos even when all the anthers had been removed, and a subsequent cytological study by MURBECK and JUEL showed that the embryos developed from the egg without fertilization. A recent study by ROSENBERG[25] shows that in the form called *Taraxacum confertum* a typical tetrad of megaspores is formed from the megaspore mother cell, and that the reduced number of chromosomes is 8, which can be counted in an early stage as prochromosomes. JUEL had reported 12 or 13 as the reduced number and 26 as the diploid number in apogamous forms of Taraxacum.

In several forms of Rosa it has been known that embryos develop even when the anthers have been removed, but ROSENBERG is not yet able to say upon cytological evidence whether these forms are apogamous or not. In the pollen mother cells of *Rosa canina* he finds, usually, about 20 univalent chromosomes and 7 bivalent ones. During the first mitosis chromosomes become disarranged, as in Hemerocallis, and more than four pollen grains are formed from a single mother cell. He thinks that the suggestion may not be entirely unfounded that the univalent chromosomes, which normally split at the second mitosis, may split at the first, and thus show a transition to the vegetative mode of division. He gives a table showing the relatively high chromosome numbers of apogamous forms.—CHARLES J. CHAMBERLAIN.

Light and germination.—HEINRICHER[26] finds, in agreement with REMER, that the seeds of *Phacelia tanacetifolia* are greatly hindered in their germination by light. Seeds just harvested and not first dried out will not germinate at all in white light, nor in the less refrangible rays, while a considerable percentage germinate in darkness and a smaller percentage in the more refrangible rays. A period of drying, whether it occurs in darkness or light, greatly increases germination in darkness and in the more refrangible rays, while it leads to a low percentage of germination in white light and in the less refrangible rays. The behavior of these seeds toward light is in most respects just opposite to that of the seeds of *Veronica peregrina*.

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[24] WENT, F. A. F. C., The inadmissibility of the statolith theory of geotropism. Proc. Koninkl. Akad. Wetens. Amsterdam **1909**:343–345.

[25] ROSENBERG, O., Ueber die Chromosomenzahlen bei Taraxacum und Rosa. Svensk. Bot. Tidskrift **3**:150–162. *figs.* 7. 1909.

[26] HEINRICHER, E., Keimung von *Phacelia tanacetifolia* Benth. und das Licht. Bot. Zeit. **67**[1]:45–66. 1909.

The stored food in the seeds of *Phacelia tanacetifolia* is largely fat. HEINRICHER argues that germination is hindered by light because acid formation is greatly fostered by darkness of by the more refrangible rays, which create more favorable conditions for the formation and action of lipase. There seems to be little evidence offered for this conclusion. In fact it looks as if our knowledge of the germination processes must be greatly extended before we can announce any one process that must be stimulated to induce germination. However this may be, it is certain that such conclusions, if tenable, must have far more experimental evidence than HEINRICHER has offered.—WILLIAM CROCKER.

Suspended life.—BECQUEREL reports to the Paris Academy of Science[27] further experiments on the question of the life of seeds, whether it is slow or stopped. He perforated the seed coats of seeds of lucerne, white mustard, and wheat, dried them in a vacuum with BaOH at 40° C. for six months, sealed them in a glass tube exhausted to 0.002^{mm} mercury, and kept them for a year; they were then submitted to a temperature of liquid air (−190°) for three weeks, and without warming up to the temperature of liquid hydrogen (−250°) for 72 hours. On being kept upon moist cotton at 28° all except one grain of wheat out of five germinated in a perfectly normal fashion. BECQUEREL finds it impossible to conceive of "life" under the conditions named, and holds that life can be interrupted completely with no prejudice to its resumption.—C. R. B.

Individual variation.—An elaborate paper upon the individual differences in the development of growing plants, with special reference to the influence of external conditions, has been published by KORIBA.[28] It is too detailed for any intelligible summary, but its data should be considered by those who are conducting experiments of any kind in which a limited number of plants furnish the basis for conclusions. It emphasizes strongly the necessity of taking account of these individual peculiarities.—C. R. B.

Respiration and temperature.—KUYPER reports[29] that BLACKMAN'S theory of limiting factors holds good for respiration, which as a chemical process agrees with the VAN'T HOFF-ARRHENIUS law between 0° and 20–25°, but shows a falling-off in an almost logarithmic curve above 40°. The "optimum" is no fixed point, for the duration of respiration at any given temperature will displace it. The course of respiration, KUYPER finds, is also dependent on the nature of the reserve food.—C. R. B.

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[27] BECQUEREL, PAUL, Sur la suspension momentanée de la vie chez certaines graines. Compt. Rend. Acad. Sci. Paris **148**:1052–1054. 1909.

[28] KORIBA, K., Ueber die individuelle Verschiedenheit in der Entwicklung einiger fortwachsenden Pflanzen mit besonderer Rücksicht auf die Aussenbedingungen. Jour. Coll. Sci. Imp. Univ. Tokyo **27**: art. 3. (pp. 86.) *pls.* 5. 1909.

[29] KUYPER, J., The influence of temperature on the respiration of the higher plants. Konink. Akad. Wetens. Amsterdam **12**:219–227. 1909.